THE MICROBIOLOGICAL BASIS OF A SOIL SUPPRESSIVE TO FUSARIUM WILT OF WATERMELON

By

ROBERT PHILIP LARKIN

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1990

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Don Hopkins for his confidence, guidance, and continued support through all stages of this research and for providing me the opportunity to pursue this degree in plant pathology.

I would also like to thank Dr. Frank Martin for his guidance, counsel, and commitment throughout this work. In addition, I would like to thank Dr. Dave Mitchell for his expertise, counsel, and encouragement when I needed it most. I also extend thanks to committee members Dr. Corby Kistler and Dr. David Sylvia for their insightful input and cooperation. Additional thanks go to Charles R. Semer IV for his expertise and assistance on various technical aspects of this work. I also thank the Richard C. Storkan Foundation for providing partial support for this work.

Special thanks are extended to my friends in Gainesville for their friendship, warmth, and comraderie which really helped me get through the rough times.

Most importantly, I want to thank my parents for their confidence, support, and patience throughout this long and winding road I have taken. Although they always wanted me to be a doctor, I don't think this is quite what they had imagined.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

THE MICROBIOLOGICAL BASIS OF A SOIL SUPPRESSIVE
TO FUSARIUM WILT OF WATERMELON

By

ROBERT PHILIP LARKIN

DECEMBER 1990

Chairman: Donald L. Hopkins
Cochairman: Frank N. Martin
Major Department: Plant Pathology

The nature of soil suppressiveness to Fusarium wilt of watermelon, caused by Fusarium oxysporum f. sp. niveum, was investigated in a unique suppressive soil developed through monoculture of watermelon cultivar 'Crimson Sweet.' The objectives of this research were to evaluate various ecological characteristics of the suppressive soil in relation to the pathogen and identify the organism(s) and mechanism(s) responsible for suppressiveness.

In addition, a method for identifying and differentiating isolates of F. oxysporum f. sp. niveum using vegetative compatibility was developed. All isolates of F. oxysporum f. sp. niveum belonged to one of three distinct vegetative compatibility groups (VCGs), and were incompatible with isolates not pathogenic on watermelon. Race 1 isolates were contained in 2 VCGs (0080 and 0081), while all race 2 isolates were contained in a third VCG (0082).

Population dynamics, root colonization, and chlamydospore germination of F. oxysporum f. sp. niveum were monitored in relation to other microorganism populations, the incidence of Fusarium wilt, and the planting of watermelon cultivars in four different suppressive and conducive soils. An orange-colored mutant isolate of F. oxysporum f. sp. niveum, comparable to the wild-type pathogen in growth, pathogenicity, and root colonization, was used to differentiate the pathogen from indigenous populations of F. oxysporum in field soils. Suppressiveness was not associated with inhibition of chlamydospore germination or increased fungistasis. Suppressive soils maintained lower pathogen populations than conducive soils, even when planted to susceptible watermelon cultivars. Successive plantings of cultivar 'Crimson Sweet' caused changes in microflora populations, including increases in bacteria, actinomycetes, fluorescent pseudomonads, and nonpathogenic F. oxysporum. Root colonization by the pathogen and other indigenous F. oxysporum was not consistently related to suppression.

Nearly 400 isolates of F. oxysporum and miscellaneous bacteria, actinomycetes, and fungi were isolated from watermelon roots and tested for their ability to reduce disease in microwave-treated and field soils. Specific isolates of nonpathogenic F. oxysporum were the only organisms consistently effective in reducing disease (35-75% reduction) in both soils. Isolate effectiveness was not related to the level of colonization of roots. The mechanism of suppression is still not clear, but may involve induced resistance.

CHAPTER 1
INTRODUCTION

Fusarium oxysporum (Schlect.) Snyd. & Hans. is an ubiquitous soil-inhabiting fungus, consisting of saprophytic as well as plant-pathogenic strains. Pathogens within this species are responsible for a severe and economically important vascular wilt disease on a staggeringly diverse group of plants and can be subdivided into numerous formae speciales, which are specialized pathogens of specific plant genera or other plant groups. These pathogens are capable of infecting the roots of susceptible host plants and extensively colonizing the xylem tissue, ultimately resulting in severe internal water stress producing wilt and eventually causing the death of the plant (Beckman, 1987; MacHardy and Beckman, 1981; Nelson, 1981). Fusarium oxysporum has no known sexual stage, but produces three types of asexual spores. Macroconidia and microconidia are thin-walled spores that serve to spread the fungus within as well as outside the host plant. Chlamydospores are thick-walled resting structures that can survive for long periods in the soil as dormant propagules in the absence of a suitable host plant (Nelson, 1981).

Fusarium oxysporum f. sp. niveum (E.F.Sm.) Snyd. & Hans., causal agent of Fusarium wilt of watermelon (Citrullus lanatus [Thunb.] Matsum. & Nakai), is pathogenic only on watermelon and occurs throughout the watermelon-growing regions of the world. Because of the severity of this disease and the ability of the pathogen to survive many years in the soil, Fusarium wilt is often a limiting factor in watermelon production.

Control of Fusarium wilt of watermelon is currently dependent upon the use of effective wilt-resistant cultivars and long rotation periods between watermelon crops (Elmstrom and Hopkins, 1981; Hopkins and Elmstrom, 1984). Resistance to the common race 1 of the pathogen is attributed to a single dominant gene (Henderson et al., 1970; Netzer and Weintall, 1980). Resistance is not complete, however, and forms a continuum ranging from susceptible to highly resistant cultivars. Many of the most highly resistant varieties are not used by commercial growers because of other undesirable characteristics (Elmstrom and Hopkins, 1981). Crop rotation is necessary even with cultivars classified as highly resistant, which often succumb to disease in heavily infested fields (Elmstrom and Hopkins, 1981; Hopkins and Elmstrom, 1984; Hopkins et al., 1987). The recent discovery in the United States of the highly aggressive race 2 (Bruton et al., 1988; Martyn, 1987), to which there is presently no known resistance, represents an additional threat to the commercial cultivation of watermelon. For these reasons current control methods of using available resistant varieties in long rotations are not always effective or practical, and alternative control measures are needed.

Soils that are naturally suppressive to Fusarium wilt diseases of numerous crops are known to occur in many regions of the world (Baker and Cook, 1974; Cook and Baker, 1983; Schneider, 1982; Toussoun, 1975). In these soils, disease does not readily develop even though the pathogen and susceptible hosts are present (Cook and Baker, 1983; Schneider, 1982). Among the most well-known and studied examples of Fusarium wilt-suppressive soils are those of the Chateaurenard region of France, where susceptible vegetables have been grown for centuries with no wilt problems (Alabouvette, 1986; Alabouvette et al., 1979, 1985b; Louvet et al. 1981), and in the Salinas Valley of California, where wilt also does not develop despite years of continuous cropping of susceptible

plants (Scher and Baker, 1980, 1982; Smith, 1977; Smith and Snyder, 1972; Sneh et al., 1984; Yuen et al., 1985).

The cause of disease suppression in these and many other such soils has been determined to be biological in origin, with suppressiveness being eliminated with aerated steam treatments at 55-60°C for 30 min, fumigation with methyl bromide, or gamma irradiation (Alabouvette, 1986; Cook and Baker, 1983; Louvet et al., 1981; Scher and Baker, 1980). Suppression has often been associated with inhibition of chlamydospore germination and reduced saprophytic growth of the pathogen (Alabouvette, 1986; Alabouvette et al., 1985a, 1985b; Huang et al., 1988; Hwang et al., 1982; Louvet et al., 1981; Scher and Baker, 1980, 1982). These soils generally have many characteristics in common, including their physical-chemical makeup (high pH, high organic matter content, and a high montmorillonite clay content), transmissibility of suppressiveness to certain other soils (as little as 1% suppressive soil needed in some cases), and effectiveness against a number of F. oxysporum formae speciales (Alabouvette, 1986; Alabouvette et al., 1979; Cook and Baker, 1983; Louvet et al., 1979; Schneider, 1982). Biological components from such suppressive soils may provide an alternative or supplemental form of wilt disease control.

Disease suppression in these soils has often been shown to result from specific types of antagonistic organisms acting in conjunction with a large, diverse population of microorganisms supported by the physical characteristics of the soils, thus providing both a specific and general suppressive effect (Alabouvette, 1986; Alabouvette et al., 1985b; Cook and Baker, 1983; Louvet et al, 1981; Scher and Baker, 1980, 1982). The specific suppression in Chateaurenard soils, as well as several other wilt-suppressive soils, has been attributed to nonpathogenic strains of F. oxysporum and F. solani (Alabouvette,

1986; Alabouvette et al., 1985b; Louvet et al., 1981; Schneider, 1984; Tamietti and Pramotton, 1990), whereas suppression in the Salinas Valley and other soils has been more closely associated with certain strains of fluorescent species of Pseudomonas (Elad and Baker, 1985a, 1985b; Scher and Baker, 1980, 1982; Sneh et al., 1984). Numerous other organisms have also been reported as causing or contributing to suppressiveness in various soil systems, including species of Arthrobacter (Smith, 1977; Sneh, 1981), Alcaligenes (Yuen and Schroth, 1986), Trichoderma (Lin and Cook, 1979; Locke et al., 1985; Marois et al., 1981), Penicillium (Lin and Cook, 1979; Marois et al., 1981), Bacillus (Yuen and Schroth, 1986), Serratia (Sneh, 1981; Sneh et al., 1985), Hafnia (Sneh et al., 1985), and others. Generally, these Fusarium wilt-suppressive soils are considered to be of the long-standing, naturally occurring type (Alabouvette, 1986; Alabouvette et al., 1985b; Cook and Baker, 1983; Hornby, 1983; Louvet et al., 1981; Shipton, 1977); their suppressiveness is inherent in the physical-chemical and microbiological structure, was present before cultivation began, and is independent of any cropping practices of susceptible or resistant plants.

In recent years, Hopkins and co-workers (1985, 1987) have reported the development of a different type of Fusarium wilt-suppressive soil in Florida. This suppressive soil was induced as a result of monoculture of a particular cultivar of watermelon. Out of 10 cultivars with varying levels of resistance to wilt, only cultivar 'Crimson Sweet' promoted the development of suppressive soil with monoculture in the field (Hopkins, 1985; Hopkins et al., 1987). This apparently cultivar-specific induction of a suppressive soil was observed after 2-3 years when 'Crimson Sweet,' normally only moderately resistant to Fusarium wilt in greenhouse and field tests (Elmstrom and Hopkins, 1981; Hopkins and Elmstrom, 1984), showed significantly less wilt and higher

yields in the field than any other cultivar. By the 5th or 6th year of monoculture, wilt incidence had reached devastatingly high levels in all other cultivars and had stabilized; disease levels were as great in highly resistant cultivars as in susceptible varieties (Hopkins et al., 1987). The 'Crimson Sweet' field plots, however, have maintained extremely low levels of wilt and high yields in the field for over 10 seasons. Soil from these plots has demonstrated suppressiveness in greenhouse tests using susceptible cultivars and additions of pathogen inoculum (Hopkins et al., 1987).

Previous work with this suppressive soil demonstrated that suppression was biological in origin; it was eliminated by fumigation with methyl bromide as well as with moist heat treatments at 65-70°C for 30 min (Hopkins et al., 1987). This suppression occurs in a soil type which is normally highly conducive to disease; it is sandy, has a low pH (6.0-6.5), and a low organic matter content (<1%) (Hopkins et al., 1987). Suppressiveness was not affected by desiccation and was not readily transmissible to other soils; at least 25-50% suppressive soil mixed with conducive soil was required to initiate noticeable suppression (Hopkins and Larkin, unpublished). Other preliminary experiments indicated adjustments in the pH of the soil from pH 4 to pH 8 had no effect on suppression and selective elimination of portions of the microflora by bacterial antibiotic treatments (including penicillin, polymyxin, streptomycin, and vancomycin) also did not significantly affect suppression (Hopkins and Larkin, unpublished). The organisms responsible for this suppression, as well as the mechanisms involved, have not yet been identified.

The mechanisms active in suppressive soils are still not completely understood. Components in the soil may suppress the pathogen directly by the destruction of hyphae or propagules by lysis or parasitism, by inhibiting propagule germination, or by reducing

saprophytic growth in some other way (Baker and Cook, 1974; Cook and Baker, 1983). Most suppressive soils have a higher level of fungistasis than conducive soils, with chlamydospore germination and germ tube length of the pathogen greatly reduced (Alabouvette, 1986; Alabouvette et al., 1979, 1985b; Huang et al., 1988; Hwang et al., 1982; Louvet et al., 1981; Smith, 1977; Smith and Snyder, 1972; Sneh et al., 1984). Antagonistic microorganisms may interfere with the nutrient supply to the pathogen and effectively prevent infection. Nonpathogenic strains of F. oxysporum have been suggested to function in suppression by effectively competing with the pathogen, either saprophytically for nutrients in the rhizosphere (Alabouvette, 1986; Alabouvette et al., 1985b; Louvet et al., 1981) or parasitically for infection sites on the root (Schneider, 1984). Fluorescent pseudomonads produce a siderophore that complexes iron in the rhizosphere, making it unavailable to the pathogen, and is thought to be the mechanism of suppression in some soils (Elad and Baker, 1985a, 1985b; Kloepper et al., 1980; Kloepper and Schroth, 1981; Scher and Baker, 1980, 1982). Some fluorescent pseudomonads also produce phenazine antibiotics, which may also be important factors in suppression in some systems (Hamdan et al., 1988; Pierson and Thomashow, 1988; Thomashow et al., 1988; Thomashow and Weller, 1988). However, an active total microbial population providing intense competition for nutrients also seems to be important in many soils. Suppressiveness also may act indirectly through the induction of host resistance to the pathogen caused by prior colonization by certain soil microorganisms. Induced resistance to F. oxysporum f. sp. batatas has been demonstrated in sweet potatoes previously inoculated with nonpathogenic strains of F. oxysporum (Ogawa and Komada, 1984, 1985, 1986). Numerous other studies have shown that nonpathogenic or avirulent strains of F. oxysporum applied to roots can protect the

host from disease when challenged by a virulent strain (Biles and Martyn, 1989; Davis, 1967, 1968; Gessler and Kuc, 1982; Martyn et al., 1990; Wymore and Baker, 1982). It is also possible that more than one mechanism may be operating in a particular suppressive soil.

Various biological components from suppressive soils have been and are being studied for their potential to control Fusarium wilt and other diseases. Specific antagonistic organisms have been isolated, screened, and tested for their ability to reduce disease in the greenhouse and in the field. However, because of the complex nature of such suppressive soils, in addition to identifying the organisms responsible, it is important to study the ecology of these soils and the specific conditions, processes, interactions, and mechanisms which make them suppressive (Baker and Chet, 1982; Cook and Baker, 1983; Toussoun, 1975). A thorough understanding of the ecological interactions of the pathogen and other microorganisms may be vital to the effective utilization of specific antagonists from these soils as biological control agents.

The unique features of the 'Crimson Sweet' suppressive monoculture soil from Florida provide an excellent opportunity for analyzing the ecological characteristics, organisms, and mechanisms responsible for suppression in this soil system. The cultivar-specific induction of suppressiveness and its development in a soil type which is normally highly conducive to wilt distinguishes this soil from most other Fusarium wilt-suppressive soils, and makes this soil especially applicable for comparative analysis with similar conducive soils.

The overall objectives of this research were to determine the nature of the microbiological basis of this suppressive soil and to provide a better understanding of how this cultivar-induced suppressive soil works. This work was undertaken as the initial

step in the development of a potential biological control system incorporating components or mechanisms from this soil. The more specific objectives were to (1) evaluate various ecological characteristics of the suppressive soil in relation to the pathogen, (2) identify the organism(s) responsible for this suppression, and (3) identify the mechanism(s) responsible.

In the past, ecological and epidemiological studies with F. oxysporum have been severely limited by the fact that particular pathogenic formae speciales are morphologically indistinguishable from other formae speciales and saprophytic strains of F. oxysporum which occur abundantly in most soils. In this study, precise identification, differentiation, and clarification of the different races of the pathogen, as well as gaining an understanding of their distribution and role in suppressive and conducive soil, may be important for understanding the mechanism of suppression. Therefore, an additional preliminary objective of this research was to develop a relatively quick, accurate, and efficient technique for identifying and distinguishing strains of F. oxysporum f. sp. niveum from those not pathogenic to watermelon, as well as differentiating pathogenic races within F. oxysporum f. sp. niveum.

CHAPTER 2
VEGETATIVE COMPATIBILITY WITHIN *FUSARIUM OXYSPORUM* F. SP. *NIVEUM* AND ITS RELATIONSHIP TO VIRULENCE, AGGRESSIVENESS, AND RACE

Introduction

Fusarium oxysporum Schlecht. f. sp. *niveum* (E.F.Sm.) Snyd. & Hans., the causal organism of Fusarium wilt of watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai), is widespread throughout the watermelon-growing regions of the world. This forma specialis is pathogenic only on watermelon and has been subdivided into two or three pathogenic races according to virulence on cultivars of varying levels of resistance (Crall, 1963; Elmstrom and Hopkins, 1981; Netzer, 1976; Netzer and Weintall, 1980). However, these races are not clearly defined and there has been difficulty in their differentiation (Armstrong and Armstrong, 1978; Martyn, 1987; McKeen, 1951).

Crall (1963) originally described two races of *F. oxysporum* f. sp. *niveum* in Florida; following the numbering system of Cirulli (1972), these are designated races 0 and 1. In general, both races caused severe wilt on susceptible cultivars, but race 0 did not wilt resistant cultivars while race 1 caused slight to moderate wilt on most cultivars classified as resistant to Fusarium wilt. Armstrong and Armstrong (1978), however, concluded that differences between these strains were not great enough to constitute distinct races and considered them all to be race 1. In 1976, Netzer (1976) described a highly aggressive race in Israel capable of causing severe wilt in all known resistant

cultivars; it was designated race 2. Within the last few years race 2 has been reported in the United States, but only in Texas (Martyn, 1987) and Oklahoma (Bruton et al., 1988).

Traditionally, F. oxysporum f. sp. niveum can be distinguished from other formae speciales and saprophytic strains of F. oxysporum only by its virulence on watermelon. Distinguishing races require screening of pathogenic isolates on cultivars of varying levels of resistance. These tests are laborious and often inconsistent or inconclusive. Results can be greatly influenced by environmental factors, host age, inoculum level, and inoculation methods (Bosland et al., 1988; Latin and Snell, 1986; Martyn and McLaughlin, 1983).

An alternative approach to the classification of strains of F. oxysporum is based on vegetative compatibility (Correll et al., 1987; Puhalla, 1985). Strains that are capable of forming heterokaryons with each other are vegetatively compatible and all such compatible strains comprise a distinct vegetative compatibility group (VCG). Since F. oxysporum lacks a sexual stage, genetic interaction is thought to be restricted primarily to strains that are vegetatively compatible with one another. Puhalla (1985) suggested that each VCG may represent a genetically isolated population, resulting in specific genetic traits being associated with particular VCGs. Using complementary nitrate-nonutilizing mutants to detect hetrokaryon formation, Puhalla demonstrated that particular VCGs always correspond with formae speciales and often with races within formae speciales. Subsequent studies have used vegetative compatibility to define, subdivide, or differentiate strains and pathogenic races in F. oxysporum f. sp. apii (Correll et al., 1986a; Ireland and Lacy, 1986), f. sp. pisi (Correll et al., 1985), f. sp. conglutinans (Bosland and Williams, 1987), f. sp. cubense (Ploetz and Correll, 1988), f. sp. melonis (Jacobsen and Gordon, 1988), f. sp. lycopersici (Elias and Schneider, 1987), f. sp. vasinfectum (Katan

and Katan, 1988), f. sp. dianthi (Katan et al., 1989), and f. sp. asparagi (Elmer and Stephens, 1989). It has also been used to differentiate nonpathogenic forms (Correll et al., 1986b; Elias and Schneider, 1987).

The objectives of this study were to determine the potential for using vegetative compatibility to distinguish F. oxysporum f. sp. niveum from other strains of F. oxysporum as well as in the differentiation or clarification of pathogenic races or other subdivisions within F. oxysporum f. sp. niveum.

Because terms such as "virulence" and "aggressiveness" have been used differently by various authors, it is necessary to clarify how these terms have been defined here. In this paper, the concepts of virulence and aggressiveness as discussed by Vanderplank (1975, 1978) and Caten (1987) are used. Virulence is considered a qualitative trait of the pathogen associated with vertical resistance in the host. Thus, virulence refers to the ability of a pathogen to cause disease on a particular host cultivar and is related to race. Aggressiveness, on the other hand, refers to the level or degree of pathogenicity, a quantitative trait, measured by disease severity on a number of different host cultivars. Aggressiveness varies independently of the differences in host cultivars and is related to horizontal resistance. Also, for the purposes of this paper, all references to pathogens or pathogenicity relate only to the ability to cause Fusarium wilt in watermelon and not to pathogens causing disease on any other host. A preliminary report of this work has been published (Larkin et al., 1988).

Materials and Methods

Isolates of Fusarium oxysporum

Initial tests for establishing vegetative compatibility groups were made using Florida isolates from a previous study (Hopkins et al., 1987), which had been tested for pathogenicity and identified as either F. oxysporum f. sp. niveum or miscellaneous isolates of F. oxysporum not pathogenic on watermelon. These included 27 isolates of F. oxysporum f. sp. niveum collected from stems of naturally infected watermelon plants as well as 25 isolates of F. oxysporum collected from field plot soils in Florida. For the present study, an additional 180 isolates of F. oxysporum were collected from the same field plot soils. Isolates of F. oxysporum f. sp. niveum originating from various locations around the world were obtained from individuals and culture collections (Table 2-1).

Fungal isolations were made from soil by adding a 5.0-g subsample to 45 ml sterile water and stirring vigorously for 10 minutes. While being stirred, 1 ml of this suspension was extracted and added to 9 ml 0.1% water agar and agitated using a vortex mixer. One milliliter of this suspension was pipetted onto each of five plates of Komada's (1975) Fusarium-selective medium and spread over the surface by tilting the plates. Isolates were identified as F. oxysporum by their characteristic growth and morphology on this medium 7-10 days later. All isolates were single-spored and cultured at 26°C on potato dextrose agar (PDA) or a minimal medium (MM) containing nitrate as its only nitrogen source (Puhalla, 1985). Isolates were stored on sterile filter paper at 4°C (Correll et al., 1986a).

Recovery of Nitrate-Nonutilizing Mutants

Methods similar to those developed by Puhalla (1985) and Correll et al. (1987) were used. A mycelial transfer (8-mm^3 PDA block) of each isolate of F. oxysporum was placed on a chlorate-amended medium of either PDA or MM containing 1.5% $KClO_3$ (Puhalla, 1985). Initially, colonies were greatly restricted, but after a few days fast-growing sectors emerged from most colonies. Portions of these sectors were transferred to MM. Colonies producing a characteristically thin expansive growth with no aerial mycelium on this medium were considered to be nitrate-nonutilizing (nit) mutants. All nit mutants were resistant to chlorate and showed wild-type growth on PDA. Numerous mutants were generated for each isolate. When paired on MM, complementary mutants were identified by the production of a distinct line of thick wild-type growth where the two colonies came in contact. This was the visual indication of hyphal anastomosis and heterokaryosis.

Vegetative Compatibility Tests

To establish VCGs, two complementary nit mutants derived from each isolate were paired in all combinations with complementary mutants of other isolates. Pairings were made by placing mycelial blocks 1.5 cm apart on MM and incubating for 7-14 days at 26°C. Compatible isolates produced a heterokaryotic tuft of wild-type growth between complementary mutants. Incompatible isolates produced no reaction. After distinct vegetative compatibility groups were established, two pairs of complementary nit mutants were selected from each VCG for use as tester strains and were used to screen all subsequent isolates for compatibility. Mutants to be used as testers were classified according to their nitrogen utilization phenotype. Each tester pair consisted of one NitM and one nit1 or nit3 phenotypic mutant as designated and defined by Correll et al.(1987).

To screen numerous isolates quickly and efficiently, a multiple-cross plating technique was used to maximize the number of crosses per plate. Using a color-coded template, six different isolates could be crossed with up to seven tester isolates in all combinations. All pairings were made at least twice using two different tester strains from each VCG.

Pathogenicity Tests and Race Determinations

Pathogenicity tests were conducted with all isolates. Initial tests were conducted using susceptible watermelon cultivars ('Florida Giant' or 'Sugar Baby') to separate pathogenic from nonpathogenic isolates. Subsequent virulence tests with pathogenic isolates to determine race designations used six cultivars, ranging in order from susceptible to highly resistant as follows: 'Florida Giant,' 'Charleston Gray,' 'Crimson Sweet,' 'Sugarlee,' 'Dixielee,' and 'Calhoun Gray.' Race determinations were made on all imported isolates and selected Florida isolates representative of each VCG. Inoculum consisted of microconidial suspensions harvested from 5- to 7-day-old PDA cultures. Conidia were added at the rate of 5×10^3/g field soil which had been microwave-irradiated (2450 MHz, 700 watts) for 2 min/kg soil at a matric potential of -0.01 MPa. This treatment was effective in eliminating indigenous F. oxysporum propagules in preliminary tests. The soil used in these tests was of the Chipley sand soil series, which are thermic, coated Aquic Quartzipsamments; it had a pH of about 5.5, cation exchange capacity of 12-16 meq/100 g soil, and an organic matter content of about 2% in the surface layers. Seeds were planted directly into the infested soil. Initial pathogenicity tests consisted of four to six replicate pots (7.5-cm diameter) of six plants each. Race determination tests used four replicate pots (10-cm diameter) of 10 plants each. Plants were maintained in a

greenhouse at 21-32°C, where maximum light intensity was 500-700 μmol/m²/s. Wilted seedlings were counted and removed weekly for 4 weeks and the percent wilt incidence was calculated. All tests were conducted at least twice.

Results

Vegetative Compatibility Tests

All isolates of F. oxysporum f. sp. niveum were found to belong to one of three distinct VCGs. Following the VCG numbering system adopted by Puhalla (1985) these are designated 0080, 0081, and 0082 (Table 2-1). Isolates which were not pathogenic on watermelon were incompatible with all isolates pathogenic on watermelon. All isolates within a VCG produced unambiguous compatible reactions with all other isolates of that VCG and no isolates were compatible with isolates from more than one VCG. All pathogenic isolates were self-compatible (Jacobsen and Gordon, 1988).

All of the imported isolates, with the exception of the two isolates of race 2 from Texas, were found to be within VCG 0080 regardless of geographic origin (Table 2-1). This VCG also contained many Florida isolates collected from both wilted plants and soil samples. Testing of isolates obtained from J.M.Crall showed an isolate originally designated by him as race 0 (JMC60-3) to be in VCG 0080, while isolates Crall classified as race 1 were contained in VCGs 0081 and 0082. No imported isolates were vegetatively compatible with VCG 0081, which to date consists only of isolates from Florida. Race 2 isolates from Texas were found to be in the same compatibility group (VCG 0082) as many highly aggressive Florida strains.

In screening 180 isolates of F. oxysporum from soil for vegetative compatibility, only 12 isolates were found to be compatible with one of the three established pathogenic VCGs. Ten of these were in VCG 0081 and 1 each in VCGs 0080 and 0082.

Pathogenicity Tests

All but two isolates that had been identified previously as F. oxysporum f. sp. niveum were verified to be pathogenic on watermelon, producing 50-100% wilt in susceptible cultivars. The two nonpathogenic isolates were not compatible with any of the three pathogen VCGs. Nonpathogenic isolates were incapable of systemically infecting and causing wilt in watermelon. Of the 180 isolates of F. oxysporum from soil, only the 12 isolates that were compatible with the pathogen VCGs were pathogenic on watermelon, causing 15-85% wilt in susceptible cultivars. Thus, all pathogenic isolates and only pathogenic isolates were contained in VCGs 0080, 0081, and 0082.

Race Determination

All pathogenic isolates were capable of causing substantial wilt (49-100%) in the susceptible cultivar 'Florida Giant,' but varied greatly in the amount of wilt produced in resistant cultivars (Table 2-2). Combined results of two separate virulence tests (individual test results are presented in Appendix A; Tables A-1 and A-2) with the imported isolates demonstrated the wide range of aggressiveness (7-90% wilt) observed on cultivars classified as resistant. Isolates produced varying levels of wilt on 'Charleston Gray' and 'Crimson Sweet,' with considerable overlap among isolates and no distinct differences attributable to race. Distinctions between races could only be observed on the most highly resistant cultivars. Texas race 2 isolates TX-X1D and TX-HC3 and Florida isolate CS85-4 were all vegetatively compatible and produced comparably severe levels of wilt on all cultivars, with significantly greater wilt (67-75%) on 'Dixielee' and 'Calhoun Gray' than with all other isolates tested. Isolates FG85-2 and FG85-15, both in VCG 0081, produced comparable wilt to some of the race 2 isolates, as well as significantly greater

wilt than the remaining race 1 isolates, on 'Sugarlee,' but not on 'Dixielee' or 'Calhoun Gray.'

An additional virulence test was conducted using several Florida isolates representing the three VCGs. Although generally higher levels of wilt were observed across all VCGs than in previous tests, similar differences regarding race were confirmed (Appendix A; Table A-3). The two isolates in VCG 0082 produced significantly greater wilt than all other isolates on 'Calhoun Gray.' Additional pathogenicity tests of other VCG 0082 isolates on 'Calhoun Gray' confirmed their ability to induce severe wilt on highly resistant cultivars (60-100%). Based on these virulence tests and vegetative compatibility data, the numerous Florida isolates in VCG 0082 were comparable in all respects to the race 2 isolates from Texas and were thus considered to be race 2.

Race 2 isolates separated distinctly from race 1 isolates on 'Calhoun Gray' in all tests. No other distinctions of race could be clearly differentiated, so all other isolates were considered to be race 1. In a separate test involving an isolate previously designated as race 0 (JMC60-3), no clear distinction could be made between the race 0 isolate and other weakly aggressive race 1 isolates (data not shown).

Relationship of Vegetative Compatibility Groups to Aggressiveness and Race

Isolates in VCG 0080 showed a wide range of aggressiveness, producing from fairly low to substantial wilt in resistant cultivars, yet all were distinguishable from race 2 isolates (Table 2-2). Isolates in VCG 0081 generally produced higher levels of wilt than VCG 0080 isolates, but there was some overlap with many VCG 0080 isolates. All VCG 0082 isolates caused severe wilt in all cultivars and were considered to be race 2.

Comparing the virulence data by VCG (Table 2-3) clarified this association of VCG to aggressiveness and race. Isolates in VCG 0082 averaged significantly greater wilt than did VCG 0080 and VCG 0081 isolates on 'Crimson Sweet,' 'Dixielee,' and 'Calhoun Gray' in all tests, and on 'Charleston Gray' in the imported isolate tests. Vegetative compatibility group 0081 wilt averages were significantly different from those of both VCG 0080 and VCG 0082 on 'Calhoun Gray' and 'Dixielee,' demonstrating a level of aggressiveness intermediate to that of race 2 and VCG 0080 race 1 isolates. However, in the Florida isolate test (Table 2-3), VCG 0081 wilt averages also were significantly lower (less aggressive) on 'Crimson Sweet' and 'Charleston Gray' than those of VCG 0080 and 0082. This difference in aggressiveness between VCG 0080 and VCG 0081 varied from test to test and with the isolates used, and did not appear to be consistently distinct or sufficiently reliable to constitute a race difference. Thus, as defined in this study, race 1 isolates were contained in two distinct VCGs, 0080 and 0081, while all race 2 isolates were in VCG 0082 (Table 2-4).

Discussion

Results from this study suggest a direct relationship between vegetative compatibility groups and virulence in F. oxysporum f. sp. niveum. All isolates of F. oxysporum f. sp. niveum studied were contained within three distinct VCGs, whereas isolates not pathogenic on watermelon were excluded from these VCGs. Vegetative compatibility tests were used to effectively identify isolates of F. oxysporum f. sp. niveum from within a population of miscellaneous strains of F. oxysporum; these findings were verified by pathogenicity tests. Although previously described races in F. oxysporum f. sp. niveum, such as race 0, could not always be differentiated by virulence tests, vegetative

Table 2-1. Isolates of Fusarium oxysporum f. sp. niveum used in comparative pathogenicity tests.

Isolate	Origin	Race	VCG	Source
JMC60-3	Florida	(0)1[a]	0080	J.M.Crall[b]
O-936	Maryland	1	0080	P.Nelson[c]
O-987	Maryland	1	0080	P.Nelson
O-1210	Indiana	1	0080	P.Nelson
O-1128	California	1	0080	P.Nelson
O-1130	California	1	0080	P.Nelson
O-1132	Taiwan	1	0080	P.Nelson
O-1182	Australia	1	0080	P.Nelson
O-974	Australia	1	0080	P.Nelson
18467	South Carolina	1	0080	ATCC[d]
44293	California	1	0080	ATCC
FG85-1	Florida	1	0080	Local[e]
CS85-1	Florida	1	0080	Local
FG85-2	Florida	1	0081	Local
FG85-15	Florida	1	0081	Local
FG85-20	Florida	1	0081	Local
JMC72-19	Florida	1	0081	J.M.Crall
CS85-4	Florida	2	0082	Local
CG85-15	Florida	2	0082	Local
TX-X1D	Texas	2	0082	R.D.Martyn[f]
TX-HC3-13B	Texas	2	0082	R.D.Martyn
JMC70-1A	Florida	2	0082	J.M.Crall
JMC72-8	Florida	2	0082	J.M.Crall

[a] This isolate was originally designated race 0 by J.M.Crall, but is considered to be race 1 in the present study.

[b] Central Florida Research and Education Center, Leesburg, FL

[c] Fusarium Research Center, Pennsylvania State University, University Park, PA

[d] American Type Culture Collection, Rockville, MD

[e] Author, Leesburg, FL

[f] Texas A & M University, College Station, TX

Table 2-2. Wilt development in different watermelon cultivars planted in soil infested with various isolates of Fusarium oxysporum f. sp. niveum.

Isolate	VCG	Race	Percent wilt on indicated cultivar[a]						
			FG[b]	ChG	CS	SL	DL	CalG	Mean
18467	0080	1	60a[c]	21a	37a	9a	8a	7a	24
O-1132	0080	1	62ab	26a	42abc	10ab	16ab	11a	28
O-1128	0080	1	74bc	32ab	45abcd	17abc	19ab	16ab	33
O-936	0080	1	91de	58def	43abc	23abcd	20ab	13a	41
O-1182	0080	1	78c	46bcd	41abc	29cde	37cd	18abc	41
O-987	0080	1	83cd	58def	51abcd	26abcde	25bc	18abc	43
FG85-1	0080	1	95de	53cde	50abcd	41e	17ab	14a	45
O-1130	0080	1	84cd	54cde	53bcd	33cde	30bcd	17abc	45
O-1210	0080	1	94de	62def	57cde	28bcde	30bcd	30cd	51
O-974	0080	1	89e	68ef	60de	38de	50d	27bc	56
FG85-2	0081	1	95de	38abc	42abc	57f	47d	49e	55
FG85-15	0081	1	95de	51cde	39ab	70fg	45d	42de	57
TX-X1D	0082	2	85cd	68ef	58de	76g	69e	74f	72
TX-HC3	0082	2	100e	78fg	74ef	71fg	70e	68f	77
CS85-4	0082	2	94de	90g	87f	83g	67e	75f	82
Cultivar Averages			85	52	51	40	36	31	

[a] Percent wilt indicates the incidence of Fusarium wilt after 4 weeks.

[b] Cultivars used were 'Florida Giant'(FG), susceptible to Fusarium wilt; 'Charleston Gray'(ChG) and 'Crimson Sweet'(CS), moderately resistant; 'Sugarlee'(SL), 'Dixielee'(DL), and 'Calhoun Gray'(CalG), highly resistant.

[c] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Means in each of two tests were based on four replicate pots of 10 plants each for each isolate/cultivar combination. Results of the two similar tests were combined for analysis (Individual test results are in Appendix A; Tables A-1 and A-2). Analysis was conducted on actual incidence data. Inoculum consisted of conidia added to the soil at 5×10^3/g soil.

Table 2-3. Average percent wilt in various watermelon cultivars caused by isolates of Fusarium oxysporum f. sp. niveum in three vegetative compatibility groups.

VCG	Race	Percent wilt on indicated cultivar[a]						Mean
		FG[b]	ChG	CS	SL	DL	CalG	
Imported isolate tests[c]								
VCG 0080	1	82a[d]	46a	48a	28a	25a	17a	41
VCG 0081	1	95a	44a	40a	63b	46b	45b	56
VCG 0082	2	91a	79b	75b	83b	70c	73c	78
Florida isolate test[e]								
VCG 0080	1	96a	86b	76b	75ab	54a	37a	71
VCG 0081	1	93a	59a	64a	72a	64b	49b	67
VCG 0082	2	99a	96b	90c	87b	82c	86c	90

[a] Percent wilt indicates the incidence of Fusarium wilt after 4 weeks.

[b] Cultivars used were 'Florida Giant'(FG), susceptible to Fusarium wilt; 'Charleston Gray'(ChG) and 'Crimson Sweet'(CS), moderately resistant; 'Sugarlee'(SL), 'Dixielee'(DL), and 'Calhoun Gray'(CalG), highly resistant.

[c] Values represent the combined averages of two similar tests (individual test results are in Appendix A; Table A-4). Isolates used were ATCC 18467, O-936, O-974, O-978, O-1128, O-1130, O-1132, O-1182, O-1210, and FG85-1 (all VCG 0080); FG85-2 and FG85-15 (VCG 0081); and TX-X1D, TX-HC3, and CS85-4 (VCG 0082).

[d] Means within columns in each test followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Means were based on four replicate pots of 10 plants each for each isolate/cultivar combination. Analysis was conducted on actual incidence data. Inoculum consisted of conidia added to the soil at 5 x 10^3/g soil.

[e] Florida isolate test consisted of isolates FG85-1 and CS85-1 (VCG 0080); FG85-2, FG85-15, FG85-20, and JMC72-19 (VCG 0081); CS85-4 and CG85-15 (VCG 0082). Same inoculum level and procedures were used as in the previous test.

Table 2-4. Vegetative compatibility groups and characteristics established for Fusarium oxysporum f. sp. niveum.

VCG	No.isolates	Race	Origin
0080	19	1	California, Florida, Indiana, Maryland, South Carolina, Australia, Taiwan.
0081	17	1	Florida
0082	24	2	Florida, Texas
Total	60		

compatibility groups did correspond with the two races that were identified. Within each VCG there existed a range of aggressiveness, yet there were distinct differences in wilt averages when grouped by VCG, with a progression of increasing aggressiveness from VCG 0080 to 0082.

Difficulties in the identification and differentiation of strains of F. oxysporum have long been major limitations to ecological and epidemiological studies with these important soil organisms. The genus Fusarium is well known for its variation within species and subspecies with respect to pathogenicity as well as morphology (Armstrong and Armstrong, 1978; Toussoun and Nelson, 1975). This variability, combined with the added variability and inconsistency associated with virulence tests, has rendered tenuous the classification of pathogenic races within many formae speciales.

For many pathogenic fungi, races are defined on the basis of differential cultivars, which have different genes for resistance to specific pathogenic races. However, races within F. oxysporum f. sp. niveum have not been this clearly defined. Early investigations (McKeen, 1951; Reid, 1958; Sleeth, 1934) reported variability in aggressiveness among isolates without any clear indication of distinct races. Even when races were identified, race differentiation depended upon gradations in the degree of wilt produced on various cultivars rather than unambiguous susceptible or resistant reactions (Cirulli, 1972; Crall, 1963; Martyn, 1987). Because of this, it has been suggested that true races do not exist in this forma specialis, but merely populations with variable aggressiveness (Armstrong and Armstrong, 1978; McKeen, 1951). Results of this study tend to support this contention. Differences between races were primarily those of level of aggressiveness on particular cultivars, rather than in virulence on differential cultivars. All pathogenic isolates appeared to be virulent on all cultivars, with differences only in the degree of wilt

produced. Caten (1987) considered racial classification by level of aggressiveness to be ambiguous and impractical. However, there do appear to be sufficient differences in aggressiveness between races 1 and 2 to justify continued classification into distinct groups whether or not these are classified as true races. The association of VCGs with these distinct pathogenic groups suggests that there are genetic differences between the groups and that VCGs can be used to identify and differentiate them. Two distinct groups within race 1 which could not be clearly distinguished by virulence tests were differentiated by vegetative compatibility.

Results from the virulence tests in this study demonstrated some of the problems associated with race identification. Isolates classified as race 1 caused from 0-50% wilt on cultivars considered resistant to race 1. Isolate ATCC 18467 was used in this study and also by others (Armstrong and Armstrong, 1978; Martyn, 1987) as a representative of race 1. In our tests ATCC 18467 was one of the most weakly aggressive of the race 1 isolates tested, and thus may not be the best isolate to use for comparison. It may be necessary to include several other race 1 isolates in such comparisons to account for the variation of wilt reactions found within this race (Martyn, 1987).

Martyn (1987) reported that several cultivars, including 'Calhoun Gray' and 'Crimson Sweet,' could be used to differentiate races 1 and 2. In the present study, 'Dixielee' and 'Calhoun Gray' were the best cultivars for separating races 1 and 2, whereas 'Crimson Sweet' was not very effective. Both 'Charleston Gray' and 'Crimson Sweet' appeared to be susceptible to some race 1 isolates but resistant to others. Martyn (1987) also noted that no cultivar was wholly effective in differentiating race 0 from race 1. Likewise, in this study no distinction could be made between races 0 and 1. The only isolate tested which was purported to be race 0 could not be distinguished from several

race 1 isolates. Cirulli (1972) based his distinction between these two races on a differential response to 'Charleston Gray;' such a response was not evident in this study. Although two subgroups were defined within race 1 (VCG 0080 and VCG 0081) and showed some differences in wilt reactions on resistant cultivars, these differences were not always clear and did not correspond with past descriptions of race 0. There were no isolates which were unable to cause some degree of wilt on resistant cultivars (race 0) (Cirulli, 1972; Crall, 1963). The distinction between race 0 and 1 has been disputed by others (Armstrong and Armstrong, 1978; Martyn, 1987) and there is no evidence here for classification as separate races.

Some of the differences observed between VCGs 0080 and 0081 also may be misleading. Although in some tests isolates in VCG 0081 were more aggressive than other race 1 isolates (Tables 2-2 and 2-3), these differences were based on too few isolates and do not indicate the entire range of aggressiveness observed in VCG 0081. Some of the isolates in VCG 0081 collected from the soil (not used in race determination tests) were only weakly aggressive. Overall, this VCG showed a wide range of aggressiveness among isolates. More extensive testing is needed to determine any real differences in aggressiveness between these two VCGs.

The inoculation method used in this study differed from that used in other studies (Latin and Snell, 1986; Martyn, 1987; Martyn and McLaughlin, 1983), but inconsistensies resulting from this procedural difference appeared to be minimal. In this study, soil was infested with conidial suspensions and then watermelon was seeded directly into the infested soil. This method was faster and easier than the root-dip transplant method commonly used. Since watermelons are usually direct-seeded in the field, no additional wounding or transplant shock was given to the test plants. Also, this inoculation method

allowed the pathogen to attack from the very earliest stages of development, as it would in the field. Additional tests were conducted using chlamydospores as inoculum and results comparable to those reported here were obtained (unpublished).

Virulence test results and cultivar resistance rankings from this study are in general agreement with those previously reported (Elmstrom and Hopkins, 1981; Hopkins and Elmstrom, 1984; Martyn, 1987; Martyn and McLaughlin, 1983). However, these results differ from those of Martyn (1987) regarding the resistance of 'Crimson Sweet.' In Martyn's tests, 'Crimson Sweet' consistently rates as one of the most highly resistant cultivars, equalling or surpassing 'Calhoun Gray.' In tests in this study as well as in numerous previous tests (Elmstrom and Hopkins, 1981; Hopkins and Elmstrom, 1984; Hopkins et al., 1987), 'Crimson Sweet' rates as moderately resistant (comparable with the resistance of 'Charleston Gray') in greenhouse and laboratory tests using fumigated or microwave-treated field soil. However, a much higher resistance in field situations and in field soil has been observed (Hopkins and Elmstrom, 1984; Hopkins et al., 1987). Since this appears to be a consistent and repeatable difference which only occurs in this one cultivar, it may be related to the unique ability of 'Crimson Sweet' to promote soil suppressiveness (Hopkins et al., 1987). Perhaps, Martyn's (1987) testing procedure in some way incorporates this additional level of resistance through the development of organisms active in the suppressiveness promoted by 'Crimson Sweet.' This may be related to the background microbial populations present or the type of soil medium and seedling transplant method used.

Numerous race 2 isolates were found to occur in Florida, and this is the first report of this race in the state. Previously, race 2 had been identified in the U.S. only in Texas and Oklahoma (Bruton et al., 1988; Martyn, 1987). Most of the race 2 strains in Florida

were isolated from field plots in which resistant cultivars had been grown in a monoculture for several years (Hopkins et al., 1987). A high percentage (>90%) of the pathogens isolated from wilted plants in these plots were race 2 (VCG 0082) isolates. It appears that growing resistant cultivars will select for and increase the abundance of race 2 propagules relative to race 1 over time (Hopkins et al., 1989; Hopkins and Lobinske, 1990). Two of the isolates found to be in VCG 0082 were isolates of J.M.Crall that were collected in 1970 and 1972. Based on this and other reports of higher than normal wilt in resistant cultivars in the field, race 2 has probably been present in Florida in low populations for many years. This may explain why even resistant cultivars must be grown in long rotations in Florida (Elmstrom and Hopkins, 1981; Hopkins and Elmstrom, 1984; Hopkins et al., 1987).

Compared to other formae speciales in which vegetative compatibility has been studied, F. oxysporum f. sp. niveum appears to be one of the least complex, having few VCGs and a direct relationship between VCG and race. Thus, characterization by vegetative compatibiltiy can be implemented and determined with relative ease. Other F. oxysporum formae speciales, including f. sp. apii (Correll et al., 1986a), f. sp. conglutinans (Bosland and Williams, 1987), f. sp. vasinfectum (Katan and Katan, 1988), and f. sp. dianthi (Katan et al., 1989), also have small numbers of VCGs and relatively direct associations with race. There are some formae speciales which have numerous VCGs and more complex relationships with race, such as F. oxysporum f. sp. asparagi (Elmer and Stephens, 1989), f. sp. cubense (Ploetz and Correll, 1988), f. sp. lycopersici (Elias and Schneider, 1987), and f. sp. melonis (Jacobsen and Gordon, 1988). It appears the utility of VCGs for identification and differentiation depends on the particular formae speciales, because each has a different relationship determined by the number of VCGs,

races, and genetic lines involved. This may be a function of the age of the pathosystem. Ancient pathosystems have had a longer time to evolve, and may result in increased genetic diversity. Similarly, VCGs which are more widely distributed geographically are considered to be older than those which only occur in limited regions. If this is the case, it would appear that VCG 0080 may be the oldest and that VCGs 0081 and 0082 may have arisen more recently.

Within F. oxysporum f. sp. niveum, vegetative compatibility can be utilized as an alternative, or at least collaborative, method to distinguish pathogenic from nonpathogenic strains of F. oxysporum and to differentiate sub-forma specialis virulence characteristics. Vegetative compatibility groups were closely associated with aggressiveness and race characteristics. This supports Puhalla's (1985) hypothesis that VCGs define distinct groups with particular genetic characteristics and have important biological and pathological significance. Because of the problems in virulence tests and the tenuous nature of race relationships, vegetative compatibility groupings may allow more precise, efficient, and objective distinctions, and may provide more appropriate divisions for subdividing F. oxysporum f. sp. niveum than those based solely on virulence reactions.

CHAPTER 3
ECOLOGY OF FUSARIUM OXYSPORUM F. SP. NIVEUM IN SOILS SUPPRESSIVE AND CONDUCIVE TO FUSARIUM WILT OF WATERMELON

Introduction

Because of the complex nature of soils suppressive to plant disease, it is important, in addition to identifying the organisms responsible, to study the overall conditions, processes, and mechanisms which make them suppressive (Baker and Chet, 1982; Cook and Baker, 1983; Louvet, 1989; Toussoun, 1975). A thorough understanding of the ecological interactions of the pathogen and other microorganisms may be vital for the effective utilization of specific antagonists from these soils as biological control agents. Through the analysis of environmental differences in suppressive and conducive soils, much insight may be gained into the effect of the suppressive soil on the pathogen and on how suppression occurs. This information could then be used to study specific interactions, organisms, and mechanisms important in the suppressive response. Only after such ecological characteristics and the active mechanisms are known can strategies be developed to enhance the antagonistic interactions under the proper conditions and at the appropriate sites to enable biological control to function to its fullest.

The soil used in this study is suppressive to Fusarium wilt of watermelon and was developed through monoculture of watermelon cultivar 'Crimson Sweet.' The cultivar-specific induction of suppressiveness in this soil and its development in a soil type which is normally highly conducive to wilt, makes it especially applicable for comparative

analysis with similar conducive soils. Using four soils to represent different suppressive and conducive conditions, the objectives of this study were to monitor the population dynamics and chlamydospore germination of Fusarium oxysporum f. sp. niveum and to evaluate the colonization of watermelon roots by F. oxysporum in relation to other microorganism populations and the incidence of Fusarium wilt. A preliminary report of portions of this work has been published (Larkin et al., 1989).

Materials and Methods

Soils Used

Four soils were used throughout this research to represent different conditions of suppressiveness and conduciveness to Fusarium wilt. All of the soils were collected from field plots and adjacent areas of the experimental farm at the Central Florida Research and Education Center, Leesburg, FL. All are of the Apopka Fine Sand soil series, which are loamy, siliceous, hyperthermic Grossarenic Paleudults. They have a pH of 6.0-6.5, are low in organic matter (<1%), clay content (<3%), cation exchange capacity (4-6 meq/100 g soil), and available water capacity, and have similar physical and chemical characteristics (Hopkins and Elmstrom, 1984). They differ only in their cropping history and the resulting biology. Soils were collected in large buckets, sieved through a 0.2-cm screen, and stored in plastic bags for up to 4 months before use. The soils are designated as follows.

(1) 'Crimson Sweet' suppressive (CSS), monoculture soil is the Fusarium wilt-suppressive soil developed through monoculture to watermelon cultivar 'Crimson Sweet'. Total populations of F. oxysporum in the soil average 1-2 x 10^3 cfu/g soil, but the watermelon wilt pathogen apparently accounts for a relatively small proportion of this total

(<20%) (Hopkins and Larkin, unpublished). Addition of substantial amounts of the pathogen do not significantly increase disease incidence (Hopkins et al., 1987).

(2) 'Florida Giant' monoculture (FGM) soil is a nonsuppressive soil from the same field as CSS, but it has been monocultured to the susceptible cultivar 'Florida Giant.' High levels of wilt occur in the field, but the soil is similar to CSS in its biology and overall effects due to prolonged monoculture. Total populations of 1-2 x 10^3 cfu/g soil of F. oxysporum are similar to those in CSS soil, with pathogen populations comprising a small, but possibly larger proportion (<30%) of the total population of F. oxysporum than in CSS soil (Hopkins and Larkin, unpublished). This soil has been called nonsuppressive rather than conducive because the addition of small amounts of the pathogen cause little change in disease, suggesting this soil does have some biological buffering capacity against the pathogen and is not as conducive as fallow and other soils.

(3) Leesburg fallow, conducive (LFC) soil is from the same vicinity as the monoculture soils, but the area had not been planted to watermelon or any other crop. This represents the natural soil of the area prior to cultivation of watermelon. Total populations of F. oxysporum are lower (0.2-1.0 x 10^3 cfu/g soil) than those in the other soils and the pathogen is generally not present; the addition of low levels of the pathogen causes substantial disease.

(4) Microwave-treated 'Crimson Sweet' suppressive (CSMW) soil is the CSS soil rendered conducive by microwave-irradiation (2450 MHz, 700 watts) for 2 min/kg soil at a matric potential of -0.01 MPa. This treatment is sufficient to remove all suppressive characteristics and eliminate all F. oxysporum and most other fungi, yet leave a large bacterial biomass.

Infestation of Soils with Fusarium oxysporum f. sp. niveum and Assay of Fusarium Wilt

Isolates of F. oxysporum f. sp. niveum were obtained from naturally infected watermelon plants in a previous study (Hopkins et al., 1987). For most tests, race 1 isolate FG35-1 was used. Chlamydospores of the pathogen were used as inoculum to simulate natural conditions in the soil. Chlamydospore inoculum was produced in stock soils by the addition of a thoroughly mixed 7-day-old liquid culture, which was grown in a mineral salts solution (Netzer, 1976) and consisted mainly of chopped mycelia and microconidia, to autoclaved (60 min/kg) soil. The soil was moistened, mixed, and allowed to dry. After 4 to 8 weeks, primarily chlamydospores remained and soil dilution plate counts were made to determine their density in soil. A 5-g subsample of soil was added to 45 ml of sterile water and stirred vigorously for 5 minutes. While stirring, 1 ml of this suspension was extracted and added to 9 ml of 0.1% water agar and agitated using a vortex mixer. One milliliter of this suspension, or of a 10-fold dilution, was pipetted onto each of four plates of Komada's (1975) selective medium for F. oxysporum and spread over the surface of the agar by tilting the plates. The stock soil (containing approximately $1x10^4$ chlamydospores/g soil) was then mixed with the soil to be infested to produce the desired inoculum level (generally about 200 chlamydospores/g soil).

Watermelon seeds of susceptible cultivar 'Florida Giant' and moderately resistant cultivar 'Crimson Sweet' were planted in pots of the infested soil (generally four replications of four to six plants in four to six pots varying with individual experiments) in the greenhouse. Plants were maintained at 20-30°C and grown for four weeks; maximum light intensity was 500-700 $\mu mol/m^2/s$. Fusarium wilt was assessed by visual inspection of the plants for wilt symptoms several times a week and verified periodically by plating

surface-disinfested stem pieces on Komada's (1975) medium. Wilt was expressed as the percentage of diseased plants over the 4-week period.

Production and Characterization of Orange Mutant Pathogen Strains

To distinguish F. oxysporum f. sp. niveum added to field soils from indigenous isolates of F. oxysporum, an ultraviolet light-induced, orange-colored, mutant strain of the pathogen was produced and characterized for use as a marker organism (Elmer and Lacy, 1987; Puhalla, 1985; Schneider, 1984). Microconidia from 5- to 7-day-old potato dextrose agar (PDA) cultures of a race 1 isolate (FG85-1) of F. oxysporum f. sp. niveum were suspended in water and adjusted to 10^6 spores/ml. A petri dish containing 10 ml of this suspension was exposed to ultraviolet light (General Electric G8T5 germicidal lamp) for 20 seconds in the dark at a distance of 10 cm; approximately 95% of the spores were killed. Survivors were plated (200-500/plate) on a sorbose-based medium (ingredients/l water: agar, 20 g; sorbose, 20 g; asparagine, 2 g; K_2HPO_4, 1 g; KCl, 0.5 g; $MgSO_4 7H_2O$, 0.5 g; tergitol NP-10, 0.5 ml; and 0.2 ml of a trace element solution composed of citric acid, 5 g; zinc sulfate, 5 g; iron sulfate, 4.75 g; $Fe(NH_4)_2(SO_4)_2 6H_2O$, 1 g; copper sulfate, 0.25 g; manganese sulfate, 0.05 g; boric acid, 0.05 g; and sodium molybenate, 0.05 g in 95 ml water) and scanned for orange mutants after 6 days. Orange mutants were readily distinguished from the wild-type by their distinct orange pigment.

Growth of the orange mutant (OM) isolates was compared with that of the wild-type parent isolate by measuring radial colony growth and microconidium production on PDA as well as weight increase of mycelia in a liquid medium. Radial growth measurements were made after 3, 5, and 7 days at 26°C. After 7 days, 10 ml of sterile water were added to the plates and the surface of the agar gently scraped to loosen the

mycelia and conidia. Conidial counts of the resulting suspension were made using a hemacytometer. Mycelial PDA blocks were also transferred to flasks containing 30 ml liquid culture medium (Netzer, 1976) and the flasks incubated at 26°C for 5 days. Mycelial mats were harvested on filter paper, washed twice with deionized water and dried at 80°C for 24 hr before weighing. Each test was conducted at least twice with four replications per isolate.

Pathogenicity of the OM isolates was tested and compared with that of the wild-type parent isolate in microwave-treated soil and a conducive field soil using both conidia and chlamydospores as inoculum. Conidia were collected from 5- to 7-day-old PDA cultures and added to the soil at the rate of 5×10^3 colony forming units (cfu)/g soil. Chlamydospores were produced as previously described and added to soil to provide 200 cfu/g soil. Watermelon seeds were planted in four to six replicate pots (five seeds per 7.5-cm pot) and wilt was assayed as described above.

Root colonization by the orange mutants was compared with that of the wild-type parent isolate in microwave-treated soil infested with the pathogen. Watermelon seeds were planted (four/pot, four replicate pots/isolate) in soil infested with 5×10^3 conidia/g soil or 200 chlamydospores/g soil and grown for 3 weeks. Roots were gently removed from the soil and washed under running water to remove adhering soil particles. Roots were blotted dry, weighed, put in sterile water, and shaken at 150 rpm for 20 minutes. The resulting suspension and a 1:10 dilution were plated on Komada's (1975) medium, and colonies were counted after 5-7 days. Counts were converted to cfu/g root fresh weight. In another technique, roots were washed, separated, cut into sections and plated directly by laying the pieces lengthwise on Komada's (1975) medium, then observed for the number of orange mutant colonies/10 cm length of root.

Survival and Population Dynamics of Fusarium oxysporum in Field Soils

Chlamydospore inoculum of an OM isolate of the pathogen (FG-OR3) was added to each of the four soils at rates ranging from 2-10 x 10^2 cfu/g soil, depending on the experiment. Infested soil was maintained either at a constant matric potential of -0.1 or -0.01 MPa or under a fluctuating moisture regime of alternating wetting and drying cycles. For maintaining a constant matric potential, infested air-dry soil was moistened with deionized water to attain the desired matric potential as determined by soil moisture release curves calculated for each soil. Subsamples of 50 g of each infested soil were kept in small, weighed, plastic screwtop containers. The containers were placed, with tops loosened, in a moist chamber and incubated at 28°C. Water was added to the containers weekly to replenish the moisture content and maintain their original weights (variation in matric potential was estimated at ± 0.005 MPa). Infested soil samples for the fluctuating moisture regime tests were put in plastic pots in the greenhouse, saturated with water, and allowed to slowly dry. After about 2 weeks, the soil was resaturated and this wetting and drying process was repeated throughout 6 months. Soil samples were taken during the dry phase of the cycle. Periodically, 5-g subsamples were taken from these and used to determine the soil populations of the OM pathogen as well as indigenous F. oxysporum by soil dilution plating on Komada's (1975) medium as previously described. Populations were monitored over a 6-month period and determined as cfu/g air-dry soil. Tests were made using various initial inoculum levels, and consisted of three replications of each soil, with two samples taken per replication. The fluctuating moisture regime and -0.1 MPa constant moisture tests were repeated, whereas the -0.01 MPa test was only conducted once.

Chlamydospore Germination

Germination of chlamydospores of the pathogen in the four soils was assessed by using the buried membrane filter technique of Adams (1967), with some modifications by Alabouvette et al. (1980, 1985a). Chlamydospores were produced by growing the fungus on carnation leaf agar and suspending the macroconidia in sterile water. The suspension was incubated in the dark at 26°C for two weeks, at which time most macroconidia had converted to chlamydospores. Chlamydospore suspensions were adjusted to 2-3 x 10^4, and 1 ml of this suspension was deposited on a 25 mm millipore membrane filter (type HA 0.45 µm) with a grid on one side, by vacuum filtration.

A glucose solution was added to 100-g subsamples of the test soils at the rates of 0, 0.1, 0.2, 0.4, and 1.0 mg/g soil to give a final matric potential of -0.01 MPa. The treated soils were placed in 5.5-cm-diameter plastic pots and the chlamydospore-containing membrane filter was buried in the soil by making a slit with a spatula, gently inserting the filter, and covering the filter with soil. The pots were incubated in a moist chamber for 24 hr at 25°C. Filters were then gently removed, rinsed in deionized water, stained with trypan blue lactophenol (Adams, 1967), steamed at 70°C for 20 min, washed with clear lactophenol by vacuum filtration, and mounted in glycerin on microscope slides. Filters were subdivided into six sectors and at least 100 chlamydospores were counted per sector at a magnification of 200X. Chlamydospores were observed for germination and germ tube length. Germination tests were conducted on representative isolates of race 1 and 2, and tests were repeated at least three times for both isolates.

Root Colonization by Fusarium oxysporum

Watermelon cultivars 'Florida Giant' and 'Crimson Sweet' were planted in the four soils infested with the OM pathogen at a rate of about 200 cfu/g soil and grown 2-3 weeks in the greenhouse. Whole root samples were gently removed from the pots and adhering soil removed under running tap water. In initial experiments (surface colonization determinations) roots were not treated further. In later experiments (internal colonization determinations) roots were surface disinfested in 0.5% sodium hypochlorite for 1 min and rinsed in deionized water. All roots were then placed in empty sterile petri plates, covered with sterile water, and the roots separated and teased apart with a dissecting needle. The water was then poured off and the intact root system was embedded in agar by pouring molten Komada's (1975) medium cooled to 45°C into the plate covering the entire root. The plates were incubated at 28°C and the colonies emerging from the root were counted 3-7 days later. Colonies of the OM pathogen, indigenous F. oxysporum, and other fungi could be easily differentiated and quantified. In this way, not only numbers, but the spatial arrangement of the pathogen in relation to other fungi could be observed.

The length of the plated root systems were estimated by a modified line intersect method (Tennant, 1975). The plates containing the roots were placed on a 0.5-cm grid and the number of intersections of a root with a grid line were counted in both the vertical and horizontal directions. Root lengths in cm were determined by multiplication of the total intersections by a conversion factor of 0.393. Root systems ranged from 50-200 cm/plate. Colonization was expressed as number of colonies per 100 cm root. Each experiment was repeated and consisted of four replications of four to six root systems each.

Soil and Root Microorganism Populations

Estimates of soil and root microorganism populations were made using standard dilution-plating procedures on various general and selective culture media. Bacterial populations (rapid-growing, aerobic organisms capable of growing readily in culture) were estimated using nutrient agar and 1/10 strength tryptic soy agar. Actinomycetes were selected for on alkaline water agar, pH 10.5 (Ho and Ko, 1980). Populations of fluorescent pseudomonads as well as certain other pseudomonads were determined on selective King's medium B containing penicillin, cyclohexamide, and novobiocin (Sands and Rovira, 1970). Bacterial plates were incubated at 26°C for 3-4 days. Plates of King's medium B were examined under ultraviolet light for colonies producing diffusible fluorescent pigments. Actinomycete plates were incubated 7-10 days and total colonies counted. Fungal populations (primarily rapid-growing, spore-forming organisms) were determined on PDA containing 1 ml tergitol NP-10 and 50 mg chlortetracycline /l medium. Plates were incubated at 26°C for 5-6 days and total colonies counted.

A series of 1:10 dilutions of the initial soil suspensions were made and 0.1-ml aliquots of the appropriate dilutions were spread-plated on the various agar media. Population densities of rhizosphere and rhizoplane microorganisms were estimated from roots of 3-week-old watermelon plants. Roots were gently removed from soil and loosely adhering soil shaken free. Roots were weighed, placed in sterile water in a 1:50 (w/v) dilution, and either shaken on a rotary shaker at 200 rpm for 20 min, or put in a sonicator (Branson ultra-sonic cleaner model B-22-4) for 5 min, depending on the experiment. Appropriate 10-fold dilutions of the resulting suspensions were plated on the various agar media. In some tests, rhizoplane organisms were estimated separately from rhizosphere organisms. After the sonication procedure, the roots were removed from the suspension,

rinsed in sterile water, and triturated in a mortar and pestle. Final dilutions were plated as with the others. Populations for all organisms were expressed as log cfu/g soil (Loper et al., 1984). For most experiments, four replications of four plates each were used for each soil or root treatment. All experiments were conducted twice.

Selective Elimination of Microorganisms by Microwave Irradiation

The effects on field soils of various exposure times to microwave irradiation on the level of disease suppression (wilt), microorganism populations, and root colonization by F. oxysporum were assessed. Soils were adjusted to a matric potential of -0.01 MPa and microwave-irradiated (2450 MHz, 700 watts) for 0, 30, 60, and 90 s/kg soil. These levels were chosen after preliminary tests (levels ranging from 0-150 s/kg at 15 s intervals) showed disease suppression was eliminated in CSS soil after a microwave exposure of 90 s/kg or longer. Chlamydospore inoculum of the OM pathogen was added to the treated soils at the rate of 200 cfu/g soil. Watermelon cultivars 'Crimson Sweet' and 'Florida Giant' were planted in the soils (four replicate pots of ten plants each for each treatment) and grown for 4 weeks. Wilt was assessed and root microorganism populations and colonization were determined as described above.

Statistical Analyses

All experiments were analyzed using standard analysis of variance (ANOVA) procedures. Significance was evaluated at $P<0.05$ for all tests and mean separation was accomplished using Duncan's multiple range test. Experimental design for most tests were variations on a randomized complete block. Most simple analyses and individual factor comparisons were conducted using a one way ANOVA. Overall results of two or

more factor experiments used a two way ANOVA. Correlation or regression analyses were conducted where appropriate. All data expressed as percentages were arcsin-transformed ($\sin^{-1}\sqrt{x}$) before analysis. All computations were made using Statgraphics (STSC, Inc.) statistical computer program on a personal computer.

Results

Production and Characterization of Orange Mutant Pathogen Strains

A total of eight orange-colored mutant isolates were successfully recovered from parent isolate FG85-1 after repeated screenings with ultraviolet light. The mutation rate was in the range of 10^{-4}-10^{-5}. The eight orange mutant isolates varied considerably regarding the characteristics measured, with some isolates revealing significantly impaired capabilities (reductions of 22-89%) relative to the wild-type parent in radial growth, root colonization, and pathogenicity (Appendix A; Table A-5). Isolates FG-OR3, FG-OR6, and FG-OR8, however, equalled or surpassed FG85-1 for all characteristics measured in initial tests and were found to be indistinguishable from the wild-type in further evaluations for root colonization and pathogenicity (Table 3-1). On the basis of these tests, it was concluded that these isolates could be used to adequately represent F. oxysporum f. sp. niveum in field soils and greenhouse tests. The orange pigment was found to be a stable and reliable marker throughout all phases of this research. Isolate FG-OR3 or FG-OR8 was used in all subsequent experiments involving an orange mutant (OM) pathogen.

Survival and Population Dynamics of Fusarium oxysporum in Field Soils

Differences in the population dynamics of F. oxysporum f. sp. niveum were observed among the soils regardless of moisture conditions or initial pathogen inoculum

levels (Figure 3-1). At constant matric potentials (-0.1 and -0.01 MPa), populations remained stable, at or near initial inoculum levels (200-600 cfu/g soil), in both monoculture soils (CSS-suppressive and FGM-nonsuppressive) throughout a 6-month study period. In the fallow conducive (LFC) soil, populations increased somewhat within the first few weeks, then remained stable throughout the remainder of the experiment. In the microwave-treated soil (CSMW), there was a dramatic initial increase in propagule numbers followed by a slow decline. Pathogen populations were higher in CSMW soil than in others throughout the study. Populations in the monoculture soils were also lower than in LFC soil at most sampling dates. In soils under a fluctuating moisture regime, represented by alternating wetting and drying cycles, an initial increase in propagules was followed by a gradual decline throughout the six months in all soils. As with constant moisture potential soils, the microwave-treated soil had the greatest increase in propagules and this difference occurred throughout the study period.

Chlamydospore Germination

There were no differences observed for chlamydospore germination in CSS, FGM, or LFC soils at any level of glucose amendments (0-1.0 mg/g soil) (Figure 3-2). Chlamydospores germinated readily (20-80%) in all soils with small additions of glucose (0.1-0.4 mg/g soil). The only difference among soils was at the 0.1 and 0.2 mg glucose/g soil level with isolate CS85-4 and at 0.2 mg/g soil with isolate FG85-1, where CSMW soil resulted in greater germination than any of the field soils. There was some variation between the different pathogen isolates tested at the lower glucose levels, with isolate CS85-4 germinating more readily than FG85-1 at 0.1 and 0.2 mg glucose/g soil, but overall effects were similar. Observational data did not indicate any differences or large

variation in germ tube lengths or in the lysis of hyphae between the three field soils at any glucose level.

Root Colonization by Fusarium oxysporum

Surface colonization levels of 'Crimson Sweet' and 'Florida Giant' watermelon roots by added F. oxysporum f. sp. niveum (OM pathogen) were similar in CSS, FGM, and CSMW soil, whereas colonization was greater in LFC soil than in the other three soils (Table 3-2). Colonization by indigenous F. oxysporum was similar in CSS, FGM, and LFC soils. There was virtually no colonization in CSMW soil due to the elimination of F. oxysporum by microwave treatment. Colonization by F. oxysporum f. sp. niveum or other F. oxysporum followed no discernible pattern of spatial arrangement on the roots. Colonization by the OM pathogen and other F. oxysporum were intermixed somewhat randomly over the surface. There was no indication of preferential colonization of root tips or young roots; colonization occurred uniformly over all root types. The ratio of colonization by F. oxysporum f. sp. niveum to colonization by F. oxysporum in CSS soil was not different from that in FGM soil for either cultivar 'Florida Giant' or 'Crimson Sweet,' but the CSS soil ratio was less than for LFC soil for cultivar 'Crimson Sweet.' Level of disease (% wilt), however, was lower in CSS soil (6.6%) than all other soils planted to 'Crimson Sweet' (47-58%). When planted to 'Florida Giant,' CSS soil produced lower wilt (31.8%) than the conducive soils (80.3 and 64.8% for LFC and CSMW soil). The level of Fusarium wilt was not related to the degree of colonization by the OM pathogen, indigenous F. oxysporum, or ratio of OM pathogen/other F. oxysporum. Overall, similar colonization levels were observed on 'Florida Giant' and 'Crimson Sweet' cultivars. Soil populations of the pathogen at the time of root sampling were lower in the monoculture

soils than in the conducive soils. With the exception of CSMW soil, which appeared to have unusually low OM colonization in this test compared to subsequent tests, colonization by F. oxysporum f. sp. niveum appeared to be related to F. oxysporum f. sp. niveum populations in the soil rather than to differential colonization in different soils or cultivars.

Since surface colonization appeared to correspond primarily with soil populations, internal colonization (or its approximation) was measured in some tests by plating surface-disinfested watermelon roots. In these tests, in which initial OM inoculum was 200 cfu/g soil, colonization by the OM pathogen averaged 0.91, 1.40, and 6.53 colonies/100 cm root for CSS, FGM, and LFC soils, respectively, while the corresponding values for colonization by indigenous F. oxysporum were 10.83, 14.92, and 5.23 colonies/100 cm root. There was no difference (Duncan's multiple range test, $P<0.05$) between CSS and FGM soils for colonization by F. oxysporum f. sp. niveum, F. oxysporum, or in their colonization ratio, although there was a difference between LFC and the two monoculture soils for all three values.

Soil and Root Microorganism Populations

Soil population estimates of general groups of microorganisms in the four soils prior to planting show some overall similarities as well as a few notable differences among the soils (Figure 3-3A). Bacterial populations were similar, ranging from 6.90 to 7.15 log cfu/g soil in the four soils. Estimates of actinomycete populations were significantly greater in the two monoculture soils (6.5 and 6.42 log cfu/g soil, for CSS and FGM, respectively) than in the conducive soils (5.44 and 6.09 log cfu/g soil for LFC and CSMW, respectively). Fluorescent pseudomonad populations were higher in CSS than any other

soil. Non-fluorescent pseudomonad-like bacteria populations also were greater in CSS than in FGM and CSMW soils. Fungal populations were highest (and showed the greatest diversity in colony morphology) in LFC soil than any of the other soils (5.42 log cfu/g soil vs. 4.94, 4.75, and 4.48 log cfu/g soil for CSS, FGM, and CSMW soils, respectively). Although actual numbers fluctuated from one test to another, differences among the soil population estimates relative to one another were similar in subsequent tests.

Estimates of microorganism populations isolated from watermelon roots also were different among the four soils (Figure 3-3B). Root populations of bacteria, actinomycetes, fluorescent pseudomonads, and other pseudomonads were greater in both monoculture soils (CSS and FGM) than in LFC or CSMW soil. Fluorescent pseudomonad populations on roots in CSS soil were also greater than in FGM soil (6.00 log cfu/g root in CSS soil and 5.35 log cfu/g root in FGM soil). Fungal populations were greater on roots in LFC than any other soil. Microorganism populations were consistently greater on roots than in soil for all prokaryote groups, with populations averaging 1-2 orders of magnitude greater per gram of roots than per gram of soil. Differences in microorganism populations between soils also tended to be greater on roots than in the bulk soil. Overall fungal populations were comparable both in the soil and on roots, although they averaged slightly lower on roots. All population differences were observed with both 'Florida Giant' and 'Crimson Sweet' cultivars, with no substantial differences observed between cultivars (Appendix A; Table A-6).

Selective Elimination of Microorganisms by Microwave Irradiation

The level of Fusarium wilt observed in soils which had been exposed to microwave treatments of 0 s (control) and 30 s/kg soil, followed by infestation of the pathogen, was

significantly different among the three soils (Figure 3-4). These differences reflected the level of suppressiveness of the respective soils; there was less disease in CSS soil than in FGM and LFC soils and FGM soil had less wilt than LFC soil. Microwave exposures of 60 s/kg soil or less had virtually no effect on Fusarium wilt in LFC and FGM soils, while there was a change in the level of suppression in CSS soil. At 90 s/kg soil, all soils showed increased levels of Fusarium wilt, and all differences in suppressiveness between the soils were eliminated. The changes in wilt with increasing microwave exposure (linear regression analysis, $P<0.05$, $b=0.65 \pm 0.11$ and $b=0.34 \pm 0.11$ for CSS and FGM soils, respectively) were different between CSS and FGM soils.

Microwave irradiation at exposures of 60 s or less did not result in any significant change in the internal colonization of roots by F. oxysporum f. sp. niveum (Table 3-3), but the 90-s treatment allowed drastic increases in pathogen colonization in both CSS and FGM soil. Internal root colonization by F. oxysporum was not different between CSS and FGM soils at any level of microwave exposure. However, microwave treatment of 60 s did reduce ($P<0.05$) colonization by F. oxysporum in CSS soil compared to that in untreated soil (5.15 versus 13.94 colonies/100 cm root). No colonization by F. oxysporum was observed at the 90-s treatment in either soil. Generally higher levels of colonization by other miscellaneous fungi were observed in FGM than in CSS soil, but microwave exposure of 60 s or less had little effect on colonization. There was little to no colonization by other fungi observed at the 90-s exposure level. Differences between disease levels in the two soils at 0 and 30-s exposures did not appear to be related to colonization either by F. oxysporum f. sp. niveum or indigenous F. oxysporum, although the reduced level of colonization by indigenous F. oxysporum in CSS soil at the 60-s exposure did coincide with an increased level of wilt observed in that soil.

Microwave treatments of up to 90 s/kg soil had no significant effects on root population estimates of total bacteria, actinomycetes, or fluorescent pseudomonads in either soil (Table 3-4). An increase observed in this test in the population of other pseudomonads in both soils at the 90-s treatment was not observed in a repeat of this experiment, in which total root bacterial populations were relatively unaffected by microwave treatment (Appendix A; Table A-7). A decrease in fungal root populations was generally observed at the 90-s treatment, although this effect was more prominent in CSS soil than FGM soil.

Root populations of bacteria, actinomycetes, and fluorescent pseudomonads were generally higher in CSS soil than in FGM soil at the 30-s and 90-s exposure levels (Table 3-4). Root populations of fungi in FGM soil were significantly greater than in CSS soil at 30-, 60-, and 90-s exposure levels. Populations of non-fluorescent pseudomonads were greater in FGM soil at 0- and 60-s exposures. Similar population levels were observed in a repeat of this experiment, although differences between CSS and FGM were not always significant. Combined data from both tests verified the differences in populations of bacteria and fluorescent pseudomonads described here, but actinomycete populations were not different between the two soils (Appendix A; Table A-8).

Rhizoplane organism population estimates demonstrated differences between CSS and FGM soils (Table 3-5). There were some differences in populations due to microwave treatments, but no consistent trends were observed with increasing exposure time. Total bacterial populations were greater on roots in CSS soil at the 0- and 30-s treatments, as were fluorescent pseudomonad populations at the 30-s and 60-s treatments, compared to those in FGM soil. Fungal populations, however, were similar at all microwave treatments except 90 s, where CSS rhizoplane populations were greater than those in

FGM soil. There were no differences in other pseudomonad populations at any microwave treatment level (4.0-4.6 log cfu/g soil). Measurements of rhizoplane organism populations were not repeated.

Since, overall, the microwave treatments had a very limited effect on general root microorganism numbers in each soil, the average microorganism populations over all microwave treatments provided an overall comparison between the two soils (Figure 3-5). Average populations of bacteria and fluorescent pseudomonads were greater in CSS soil than in FGM soil for both rhizosphere and rhizoplane organisms, as were actinomycete rhizosphere populations. Fungal rhizosphere populations were greater in CSS soil, but rhizoplane populations were greater in FGM soil.

Discussion

Suppressive soils can be categorized as pathogen-suppressive or disease-suppressive based on whether they act directly on the pathogen to reduce its population in the soil or act indirectly by reducing the disease-causing activity of the pathogen (Cook and Baker, 1983; Hornby, 1983; Simon and Sivasithamparam, 1989). However, this distinction may not always be known or a soil may incorporate both means of suppression. Components in the soil may suppress the pathogen directly by destruction of propagules or hyphae through lysis or predation, by inhibiting propagule germination, or by reducing saprophytic growth in some other way (Cook and Baker, 1983, Schneider, 1982, Simon and Sivasithamparam, 1989). Suppression of the disease-causing activity of the pathogen may involve reduction in parasitic colonization or infection of the host or reduced ability to induce disease after infection (Cook and Baker, 1983).

Table 3-1. Comparison of selected orange mutant isolates with wild-type parent isolate (FG85-1) of Fusarium oxysporum f. sp. niveum for growth characteristics, root colonization, and pathogenicity.

Isolate	Radial growth[a] (mm)	Conidium production[b] (cfu x 10^6/ml)	Mycelium mass[c] (mg)	Root colonization[d]		Pathogenicity[e] (% wilt)	
				Rootwash (cfu x 10^3/g)	Direct (cfu/10cm)	MW-treated soil	Field soil
FG85-1	38.9[f]	1.50a	96	3.7	5.9	92	69
FG-OR3	38.0	3.52c	95	4.6	9.7	91	69
FG-OR6	36.9	1.75ab	95	4.3	-	81	62
FG-OR8	37.9	2.13b	84	8.2	9.9	86	78

[a] Radial growth was measured on PDA plates after 7 days at 26°C (Average of three experiments, each with four replicate plates/isolate).

[b] Conidium production was estimated from 10 ml conidium suspensions made from 7-day-old PDA cultures (Average of two experiments with four replications/isolate).

[c] Isolates were grown in liquid medium (Netzer, 1976) for 5 days at 26°C. Means represent the dry weight of mycelial mats harvested on filter paper (Average of two experiments with four replications/isolate).

[d] Weighed root samples (four/isolate) of cultivar 'Florida Giant' were shaken in sterile water 20 minutes and the rootwash suspension dilution-plated on Komada's (1975) medium. For direct-plating, root samples were washed, separated, cut into sections and plated lengthwise. Chlamydospore inoculum of 200 cfu/g soil was used in all tests.

[e] Pathogenicity was measured as the incidence of Fusarium wilt on cultivar 'Florida Giant' in a microwave (MW)-treated soil (2 min/kg soil at -0.01 MPa) and a conducive field soil. Chlamydospore inoculum of 200 cfu/g soil was used in all tests (Average of three tests in microwave-treated soil and two tests in field soil using four replicate pots of five plants/test).

[f] Means within columns not followed by letters or followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Figure 3-1. Population dynamics of Fusarium oxysporum f. sp. niveum over time in four soils under different moisture regimes (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment). A) Constant matric potential of -0.1 MPa; B) constant matric potential of -0.01 MPa; C) fluctuating moisture potential caused by alternating wetting and drying cycles (soils saturated and allowed to dry in 2-week cycles). Fusarium oxysporum f. sp. niveum was added to soil as an orange-colored mutant isolate. Values within tests at each sampling date denoted by the same letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

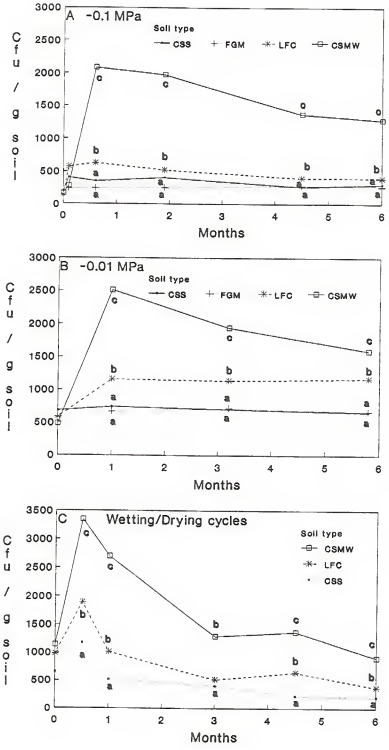

A -0.1 MPa
Soil type
CSS
FGM
LFC
CSMW
Cfu/g soil
Months
B -0.01 MPa
Soil type
CSS
FGM
LFC
CSMW
Cfu/g soil
Months
C
Wetting/Drying cycles
Soil type
CSMW
LFC
CSS
Cfu/g soil
Months

Figure 3-2. Chlamydospore germination of two different isolates of Fusarium oxysporum f. sp. niveum in four soils after additions of glucose (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment). A) Race 1 isolate FG85-1 (average of four tests); B) race 2 isolate CS85-4 (average of three tests). Values denoted by asterisk are significantly different from others at that glucose level within each isolate according to Duncan's multiple range test ($P<0.05$).

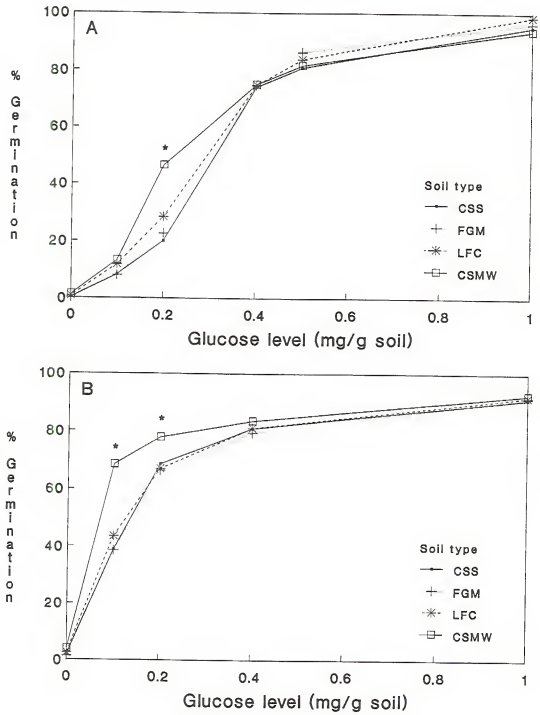

A
B
% Germination
Glucose level (mg/g soil)
Soil type
CSS
FGM
LFC
CSMW
0
20
40
60
80
100
0.2
0.4
0.6
0.8
1
*

Table 3-2. Surface colonization of roots of two different watermelon cultivars by Fusarium oxysporum in four soils in relation to disease incidence and populations of F. oxysporum f. sp. niveum.

Soil type[a]	Colonies per 100 cm root[b]		Colonization ratio[c]	% Wilt	OM pathogen population[d] cfu/g soil
	OM pathogen	F. oxysporum			
'Crimson Sweet'					
CSS	6.0a[e]	27.5b	0.215a	6.6a	44a
FGM	9.8a	33.1b	0.367ab	51.4b	50a
LFC	26.8b	21.3b	1.360b	58.0b	231b
CSMW	12.9a	0.2a	10.125c	47.1b	269b
'Florida Giant'					
CSS	8.6a	24.7b	0.518a	31.8a	62a
FGM	11.3a	27.0b	0.429a	43.8ab	38a
LFC	26.4b	17.0b	1.964a	80.3c	450c
CSMW	12.8a	0.3a	19.000b	64.8bc	238b

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive); LFC=Leesburg fallow conducive soil; CSMW=microwave-treated, 'Crimson Sweet' soil (conducive).

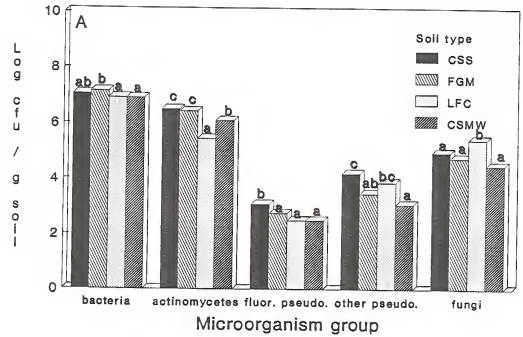
[b] Colonization of roots by the orange mutant (OM) strain of F. oxysporum f. sp. niveum and all F. oxysporum other than the OM pathogen was determined by washing the roots of 3-week-old plants and embedding them intact in Komada's (1975) medium. Four replications of four to six roots each were used. Root length was estimated by the line-intersect method (Tennant, 1975).

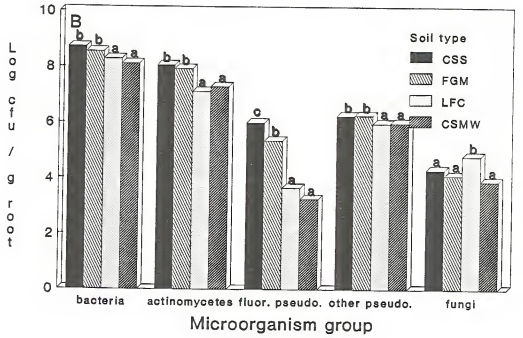
[c] The colonization ratio represents the mean of the colonization by the OM pathogen divided by the colonization by other F. oxysporum calculated for each sample.

[d] Soil population of OM pathogen was determined by dilution-plating at the time of root colonization measurements. Initial inoculum was approximately 100 cfu/g soil.

[e] Means within columns for each cultivar followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Figure 3-3. Population estimates of bacteria, actinomycetes, fluorescent pseudomonads, other pseudomonads, and fungi in soil and on watermelon roots in four different soils (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment). A) Soil microorganism populations prior to planting to watermelon; B) microorganism populations on 3-week-old watermelon roots (combined values for cultivars 'Florida Giant' and 'Crimson Sweet'). Values within each microorganism group topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.


A
Soil type
CSS
FGM
LFC
CSMW
Log cfu / g soil
10
8
6
4
2
0
a ab b a a
c c a b
b a a a
c ab bc a
a a b a
bacteria
actinomycetes
fluor. pseudo.
other pseudo.
fungi
Microorganism group


B
Soil type
CSS
FGM
LFC
CSMW
Log cfu / g root
10
8
6
4
2
0
b b a a
b b a a
c b a a
b b a a
a a b a
bacteria
actinomycetes
fluor. pseudo.
other pseudo.
fungi
Microorganism group

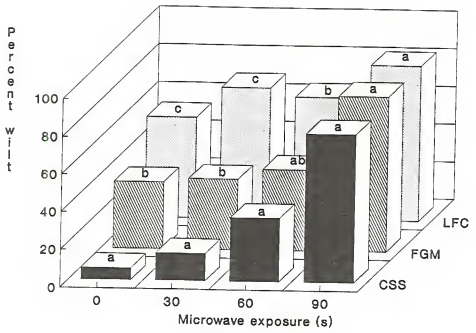


Figure 3-4. Fusarium wilt development in three soils exposed to varying length microwave exposures (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil). Chlamydospore inoculum of 200 cfu/g soil was added to each soil following microwave treatment. Values within a given exposure level topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Table 3-3. Internal colonization of 'Crimson Sweet' watermelon roots by Fusarium oxysporum in two soils exposed to varying microwave treatments.

Soil type[a]	Microwave exposure[b]	Colonies per 100 cm root			% Wilt
		OM pathogen[c]	F. oxysporum[d]	Other fungi[e]	
CSS	0	1.02a[f]	13.94b	1.37a	6.2a
FGM	0	0.55a	14.34b	5.77b	35.5bc
CSS	30	0.80a	7.72ab	1.66a	14.5ab
FGM	30	2.24a	15.51b	4.11ab	37.5c
CSS	60	0.71a	5.15a	3.26a	33.7bc
FGM	60	0.34a	8.11ab	11.11b	43.0c
CSS	90	7.48b	0.00a	0.63a	78.7d
FGM	90	14.19c	0.00a	0.00a	82.5d

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive).

[b] Duration of microwave exposure in s/ kg soil (2450 MHz, 700 watts) at -0.01 MPa matric potential. Following microwave treatment all soils were infested with the OM pathogen at 200 cfu/g soil.

[c] Colonization of roots by an orange mutant (OM) strain of F. oxysporum f. sp. niveum (FGOR-3) was determined on the roots of 3-week-old plants that had been washed, surface-sterilized in 0.5% sodium hypochlorite for 1 min, rinsed, and embedded intact in Komada's (1975) medium. Four replications of four to six roots each were used. Root length was estimated by line intersect method (Tennant, 1975).

[d] All F. oxysporum other than the OM pathogen was counted; roots were prepared as described above.

[e] All other fungi which were capable of restricted growth on Komada's (1975) medium were counted.

[f] Means in columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Additional statistical comparisons (ANOVA, Duncan's multiple range test) between the two soils at each microwave exposure level as well as within each soil over microwave exposures did not result in any significant differences other than those already indicated. Linear regression analysis of wilt data ($P<0.05$) also demonstrated a difference between the two soils with increasing microwave exposure ($b=0.65 \pm 0.11$ for CSS soil and $b=0.34 \pm 0.11$ for FGM soil).

Table 3-4. Microorganism populations on 'Crimson Sweet' watermelon roots in two soils exposed to varying microwave treatments.

Soil type[a]	Microwave exposure[b] (s/kg soil)	Log cfu / g root[c]				
		Bacteria	Actinomycetes	Fluorescent pseudomonads	Other pseudomonads	Fungi
CSS	0	8.03ab[d]	7.25ab	5.51bc	5.68a	4.40bc
FGM	0	7.68a	7.10a	5.18ab	6.23b	4.55bcd
CSS	30	8.48c	7.58c	5.54bc	6.07ab	4.26abc
FGM	30	8.03ab	7.17ab	5.04a	6.33bc	5.10d
CSS	60	7.87ab	7.29ab	5.19ab	5.70a	4.01ab
FGM	60	7.78ab	7.10a	4.51a	6.22b	5.02d
CSS	90	8.17bc	7.33b	6.24c	6.64c	3.66a
FGM	90	7.86ab	7.10a	5.16ab	6.64c	4.74cd

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive).

[b] Values represent the duration of microwave exposure (s/kg soil) (2450 MHz, 700 watts) at -0.01 MPa matric potential. Following microwave treatment all soils were infested with OM pathogen at 200 cfu/g soil.

[c] Estimates of microorganism populations were made by sonication of roots from 3-week-old plants in sterile water for 5 min and the resulting suspensions dilution-plated on various agar media. Four replications of two roots each were used. Bacterial populations were estimated on nutrient agar and 1/10 strength tryptic soy agar; actinomycete populations were estimated on alkaline water agar; pseudomonad populations were estimated on a selective King's medium B (Sands and Rovira, 1970). Fluorescent strains were identified by the production of diffusible fluorescent pigment when plates were examined under UV light. Fungal populations were estimated on potato dextrose agar with tergitol and chlortetracycline added.

[d] Means in columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Additional statistical comparisons (ANOVA, Duncan's multiple range test) between the two soils at each microwave exposure level as well as within each soil over microwave exposures did not result in any significant differences other than those already indicated. Linear regression analysis over microwave exposure was not significant ($P<0.05$) except for populations of fluorescent pseudomonads, other pseudomonads, and fungi in CSS soil ($b=0.007 \pm 0.002$, -0.016 ± 0.003, and -0.0065 ± 0.0025, respectively) and fluorescent pseudomonads in FGM soil ($b=0.0073 \pm 0.0019$).

Table 3-5. Rhizoplane organism populations in two soils exposed to varying microwave treatments.

Soil type[a]	Microwave exposure[b] (s/kg soil)	Log cfu / g root		
		Bacteria[c]	Fluorescent pseudomonads[d]	Fungi[e]
CSS	0	5.95bc[f]	4.37b	2.59abc
FGM	0	5.48a	3.40ab	2.74bcd
CSS	30	5.94bc	4.04b	2.07a
FGM	30	5.48a	2.77a	2.12a
CSS	60	6.04c	4.16b	3.22d
FGM	60	5.78abc	2.82a	2.79cd
CSS	90	5.92bc	3.77ab	3.10cd
FGM	90	5.69ab	3.69ab	2.15ab

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive).

[b] Values represent the duration of microwave exposure (s/kg soil) (2450 MHz, 700 watts) at -0.01 MPa matric potential. Following microwave treatment all soils were infested with OM pathogen at 200 cfu/g soil.

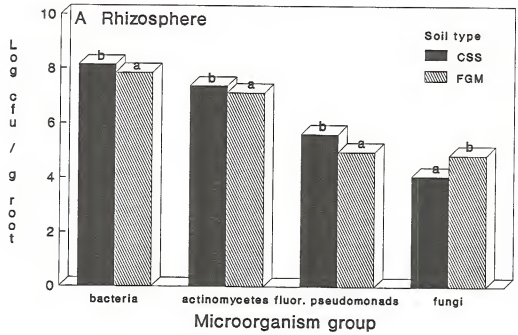
[c] Estimates of rhizoplane bacterial populations were made after sonication of roots from 3-week-old plants in sterile water for 5 min. Roots were rinsed in sterile water and triturated in sterile water with a mortar and pestle. The resulting suspensions were dilution-plated on nutrient agar and 1/10 strength tryptic soy agar. Four replications of two roots each were used.

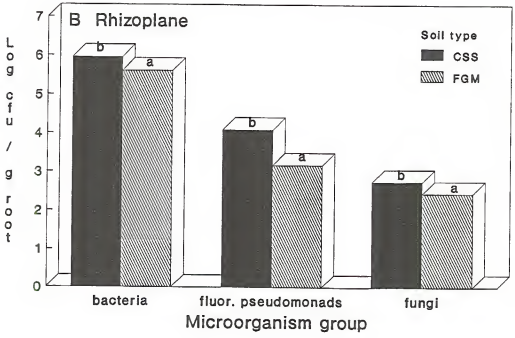
[d] Fluorescent pseudomonads were estimated on King's medium B with penicillin, cyclohexamide, and novobiocin added. Plates were examined under UV light for the production of diffusible fluorescent pigment.

[e] Fungal populations were estimated on potato dextrose agar with tergitol and chlortetracycline added.

[f] Means in columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Additional statistical comparisons (ANOVA, Duncan's multiple range test) between the two soils at each microwave exposure level did not result in any significant differences other than those already indicated. Comparisons within CSS soil over microwave exposure did indicate some additional significant differences in fluorescent pseudomonad and fungal populations among microwave exposures. Linear regression analysis over microwave exposure was not significant ($P<0.05$) except for populations of fluorescent pseudomonads and fungi in CSS soil ($b=-0.0055 \pm 0.0015$ and 0.0089 ± 0.0035, respectively) and bacteria in FGM soil ($b=0.0030 \pm 0.0013$).

Figure 3-5. Rhizosphere and rhizoplane microorganism populations on 3-week-old 'Crimson Sweet' watermelon roots in two soils (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil). A) Rhizosphere population estimates; B) rhizoplane population estimates (Averages over all microwave treatments). Values within each microorganism group topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.


A Rhizosphere
Soil type
CSS
FGM
Log cfu / g root
10
8
6
4
2
0
b
a
b
a
b
a
a
b
bacteria
actinomycetes
fluor. pseudomonads
fungi
Microorganism group


B Rhizoplane
Soil type
CSS
FGM
Log cfu / g root
7
6
5
4
3
2
1
0
b
a
b
a
b
a
bacteria
fluor. pseudomonads
fungi
Microorganism group

In this study, there was no evidence of active destruction of pathogen propagules in the suppressive soil. Populations of Fusarium oxysporum f. sp. niveum were stable over a 6-month period, with no substantial decrease in propagules over time. While the pathogen did multiply somewhat in the conducive soils (initial increases in field soil as well as microwave-treated soil), it did not multiply in the monoculture soils. This indicates some differences in fungistasis among the soils, since the ability of an organism to multiply in a soil is one of the criteria used to determine the presence or absence as well as level of microbiostasis (Ho and Ko, 1982).

Chlamydospore germination has been used as an indicator of the fungistatic activity and suppressiveness of soils (Cook and Baker, 1983; Huang et al., 1988; Hwang et al., 1982). No differences were observed in pathogen chlamydospore germination as a response to glucose amendments among the three field soils used in this study. There were no differences noted in germ tube lengths or in the lysis of hyphae. Thus, there was no indication of suppression or reduction of pathogen saprophytic growth within the suppressive soil by these measures. Higher germination was noted in CSMW soil at some glucose levels, but this soil had its microbiology, nutritional status, and chemistry seriously altered by microwave treatment, and would be expected to have had a low level of fungistasis.

No relationship was observed between suppressiveness and chlamydospore germination in these soils. This differs distinctly from most other Fusarium wilt-suppressive soils that have been studied, in which suppressiveness has been consistently associated with a high level of fungistasis, reduction of saprophytic growth, and the inhibition of chlamydospore germination (Alabouvette, 1986; Alabouvette et al., 1985b; Louvet et al. 1981; Cook and Baker, 1983; Schneider, 1982; Smith, 1977). Compared to

chlamydospore germination studies in other Fusarium wilt-suppressive soils, CSS soil required much lower levels of glucose to overcome fungistasis and initiate germination. At 0.4 mg glucose/g soil, chlamydospore germination in CSS soil was around 75-80%; whereas in several other reported suppressive soils, chlamydospore germination averaged only 10-35% at similar glucose levels (Alabouvette et al. 1980, 1985a; Hwang et al., 1982; Smith and Snyder, 1972; Sneh et al., 1984). In these other studies, germination rates in conducive soils averaged from 60-90% at around 0.5 mg glucose/g soil, indicating that fungistasis levels in soils in this study (CSS, FGM, and LFC) are uniformly low and comparable to conducive soils in other studies. Since suppression related to inhibition of chlamydospore germination is usually attributed to a general nutrient competition due to a large antagonistic microbial biomass, it appears that this general suppression evident in other suppressive soils is probably not a major factor in the CSS suppressive soil. However, since the suppressive soil did maintain lower pathogen populations and the pathogen was unable to multiply as readily as in conducive soils, saprophytic growth and development may be affected at some stage other than chlamydospore germination.

High populations of nonpathogenic F. oxysporum have been shown to be involved in suppressiveness in some Fusarium wilt-suppressive soils. Alabouvette and others (Alabouvette, 1986; Alabouvette et al., 1985b, Louvet et al. 1981) determined that the primary cause of suppression in the Chateaurenard region soils is a result of intrageneric competition in the immediate vicinity of the roots during the saprophytic development that precedes establishment of F. oxysporum at the root surface. Schneider (1984), who associated nonpathogenic F. oxysporum with suppression of celery wilt in a California soil, attributed the mechanism of suppression to parasitic competition for infection sites

on the root. Thus, he distinguished the saprophytic competition that precedes root colonization from parasitic competition for root infection. Tamietti and Pramotton (1990) attributed the suppression of flax wilt in three soils in Italy to nonpathogenic strains of F. oxysporum by a similar mechanism. Paulitz and co-workers (1987, Park et al., 1988) introduced isolates of F. oxysporum which reduced Fusarium wilt of cucumber in field soils, but the mechanism was not determined. Pathogenic F. oxysporum are known to be capable of superficially infecting and colonizing a wide number of nonsusceptible hosts without causing disease (Armstrong and Armstrong, 1948; Banihashemi and deZeeuw, 1975; Hendrix and Nielsen, 1958; Katan, 1971), and may be nearly as effective as nonpathogens in colonizing nonsuscepts and crop residues (Elmer and Lacy, 1987; Gordon et al., 1989; Gordon and Okamato, 1990). Many of the so-called "nonpathogens" in these studies may actually be pathogens of other hosts.

Other studies have shown that nonpathogenic or avirulent F. oxysporum strains applied to roots can protect the host from disease when challenged by a virulent strain (Biles and Martyn, 1989; Davis, 1967, 1968; Gessler and Kuc, 1982; Martyn et al. 1990; Ogawa and Komada, 1984, 1985, 1986; Shimotsuma et al., 1972; Wymore and Baker, 1982). Ogawa and Komada (1986) demonstrated this protection to be a result of an induced systemic resistance caused by previous infection by F. oxysporum. Although induced resistance was also implied or suggested in many other studies, it has generally not yet been proven to be the mechanism involved. In most of these studies, cut, wounded, or bare roots were dipped in concentrated conidial suspensions of the antagonist and then challenged with the pathogen in the same way with these same roots. Thus, the possibility remains that some type of competition for infection may be occurring on or within the root. Moreover, an induced resistance of the type described

has not yet been shown to occur under natural conditions and it is not known whether such a mechanism may be operating in suppressive soils (Louvet, 1989; Matta, 1989).

In this study, surface colonization and internal colonization of watermelon roots by F. oxysporum were analyzed, so that the possibility of both saprophytic and parasitic competition could be evaluated in this suppressive soil. Overall, colonization levels were similar to those reported for F. oxysporum by others (Gordon et al., 1989; Gordon and Okamato, 1990). However, neither surface nor internal colonization measurements revealed significant differences between CSS and FGM soils, although there were differences between the two monoculture soils and the two conducive soils. Surface colonization levels appeared to be affected primarily by soil population levels. Internal colonization by F. oxysporum f. sp. niveum averaged consistently lower in CSS soil, but was not significantly different than colonization in FGM soil. Differences in wilt among the soils did not appear to be related to the levels of colonization by the OM pathogen, indigenous F. oxysporum, or the ratio of OM pathogen/other F. oxysporum. Thus, saprophytic competition provided by large populations of nonpathogenic F. oxysporum, as observed by Alabouvette (1986), is apparently not the mechanism responsible for suppression in this soil.

Since colonization by the general population of F. oxysporum was not related to suppression, it could indicate that specific strains of F. oxysporum rather than general population levels may be responsible. Schneider (1984) observed that many strains of F. oxysporum were capable of infecting roots, but only some of these were effective antagonists. This may explain why the level of colonization by F. oxysporum is comparable in CSS and FGM soils, yet only CSS is suppressive. The suppressive soil may contain more isolates capable of reducing disease, which are enhanced by the

cultivar 'Crimson Sweet.' These strains may not colonize roots more effectively than others, but may be more effective in suppressing disease.

Indigenous isolates of F. oxysporum f. sp. niveum which were not marked with the orange phenotype may be present in the field soils used in these experiments. Since these would be measured as indigenous F. oxysporum, they must be taken into consideration, particularly if the numbers of indigenous pathogens differ in CSS and FGM soils. Wilt of susceptible cultivars in unamended CSS soil is consistently low (0-20%), whereas it can be quite variable in FGM soil, ranging from 10-70% wilt and averaging about 30-50% wilt in greenhouse trials (unpublished). However, disease levels in either soil often do not change substantially with addition of moderate amounts of the pathogen (up to 300 clamydospores/g soil); differences in disease levels between the soils may not necessarily be caused by different pathogen populations. Based on pathogenicity tests of limited numbers of isolates of F. oxysporum taken from the soil and roots, the proportion of indigenous pathogenic isolates of F. oxysporum f. sp. niveum relative to the total population of F. oxysporum in CSS soil is thought to be quite small (<20%), and pathogen populations in FGM soil appear to be only slightly higher (<30%) (Hopkins and Larkin, unpublished). Although it is not known what percentage of root colonization by indigenous F. oxysporum is actually due to unmarked isolates of the pathogen in these experiments, plating of surface-disinfested stems and roots from diseased plants only rarely revealed systemic infection by non-orange-mutant pathogens. Moreover, wilt in the root colonization experiments resulted from systemic infection by the OM pathogen only, so relationships between colonization and level of wilt were not affected. Thus, although a slightly larger percentage of colonization by F. oxysporum in FGM soil may have been from pathogenic strains, the effect on the overall results appears to be minimal.

Nonetheless, the possibility of parasitic competition on the root cannot be fully evaluated without additional experiments that can more accurately monitor colonization by pathogens versus nonpathogens and comparisons of specific antagonist strains.

Population estimates of total bacteria, actinomycetes, fluorescent pseudomonads, and fungi in the soil and on root surfaces were similar to those reported by others using similar soils and methodology (English and Mitchell, 1988; Simon and Sivasithamparam, 1988). Populations of these microorganism groups showed significant differences among the different soils, with fluorescent pseudomonad populations on watermelon roots and in the soil being consistently greater in CSS soil than all others. 'Crimson Sweet' suppressive soil also supported significantly greater bacteria and actinomycete root populations than the other soils in some tests. The substantially higher microorganism populations found on roots as opposed to within the soil in all soils demonstrate the importance of the root on soil microorganisms (Foster and Bowen, 1982). The greater effect of the root on bacterial populations over fungi is a result of the greater ability of bacteria to quickly colonize roots and most effectively utilize root exudates. Fluorescent pseudomonad populations showed the largest increases throughout this study, both due to soil differences and due to root influence. Fluorescent pseudomonads are known to be very effective and competitive root colonizers and have often been associated with disease suppression and promotion of plant growth (Schroth and Hancock, 1982). The differences in fluorescent pseudomonad populations between CSS and FGM soils suggest that fluorescent pseudomonads may be responding to differences in root exudates between cultivars, and thus may be important in the suppressive response.

Exposure of the soils to microwave treatments had little effect on overall microorganism numbers on watermelon roots, and there was also no difference in the

relative effect between CSS and FGM soils. This indicated that although there were differences in population levels between the soils, there was no obvious differential response due to composition of organisms which may have responded differently to heat and microwave treatment. However, these experiments did demonstrate that at the point at which populations of indigenous F. oxysporum were eliminated (90 s), suppression was lost, and where populations of F. oxysporum were suppressed markedly (60 s), disease suppression was also partially lost. At the same time, microwave exposure had no significant effect on overall numbers of bacteria, actinomycetes, or pseudomonads on watermelon roots. Overall fungal populations were also not as severely affected as F. oxysporum at the 90-s treatment. However, although overall numbers may not have been affected, species composition and diversity, which were not monitored in this study, may have been drastically altered by microwave exposure. Microwave treatments are known to have a greater effect on fungal populations than on bacterial populations, and are considered more desirable than autoclaving or fumigation for eliminating fungal pathogens because it is less disruptive and leaves a large, relatively diverse bacterial biomass (Ferriss, 1984). This is indirect evidence that F. oxysporum or some other fungi, rather than bacteria, may be important in the suppressive response.

Throughout these tests little difference has been noted between CSS and FGM soils, even though there is a large difference between these soils in the level of wilt observed in the field. When compared to conducive soils, many of the characteristics of these soils appear to be the result of monoculture and not specific differences related to cultivars, yet only CSS soil is actually suppressive, whereas monoculture soils from 'Florida Giant' and other cultivars, although not suppressive, are not as conducive as

fallow or rotation soil (Hopkins et al., 1987). Determining the significant difference between these soils is critical to understanding this suppression.

The suppressive soil used in this study had already been shown to be different from the majority of the described Fusarium wilt-suppressive soils in many ways (Hopkins et al., 1987). In the current study, many additional characteristics of the ecology of the pathogen in this soil have been analyzed, indicating additional differences from other Fusarium wilt-suppressive soils, as well as possible interactions and mechanisms important in the suppressive response. This soil appears to be disease-suppressive, rather than pathogen-suppressive, in that the disease-causing activity of infection and development of disease is where suppression most probably occurs. From this work, the organisms showing the largest differences between suppressive and conducive soils, and thus the most important organisms to study further for their interactions with the pathogen, were indigenous F. oxysporum and fluorescent pseudomonads. It is most interesting that these are the two groups most often associated with antagonism or suppression of Fusarium wilt in other soils (Cook and Baker, 1983; Alabouvette, 1986; Alabouvette et al., 1985b; Huang et al., 1988; Louvet et al., 1981; Scher and Baker, 1980, 1982). However, other organisms not yet identified from the general microorganism groups monitored in this study may also be important in suppression.

This study has dealt with general ecological characteristics and general soil and root populations of microorganisms. Cultivation to watermelon appeared to have some general effects on the microorganism populations in these soils. The key to understanding this suppression may be in specific antagonistic isolates which are different among the soils. Cultivar 'Crimson Sweet' may promote specific strains with characteristics which make them more effective as antagonists and are present in greater

numbers in CSS soil. The next step in this research will be to analyze the specific effects of successive planting of 'Crimson Sweet' on microorganism populations followed by a close look at specific antagonists and their interactions with the pathogen.

CHAPTER 4
THE EFFECT OF SUCCESSIVE WATERMELON PLANTINGS ON FUSARIUM OXYSPORUM AND OTHER MICROORGANISMS IN SOILS SUPPRESSIVE AND CONDUCIVE TO FUSARIUM WILT OF WATERMELON

Introduction

Soil suppressiveness to plant disease can occur naturally (inherent within the chemistry and biology of the soil and independent of cropping) or be induced by some practice or activity, such as planting a crop, the addition of organisms or nutritional amendments, or a particular cultural practice, that causes a change in the microflora environment (Baker and Cook, 1974; Cook and Baker, 1983; Hornby, 1983; Shipton, 1977). Induced suppressive soils are exemplified by the occurrence of take-all decline of wheat, in which suppressiveness to Gaumannomyces graminis var. tritici results after several years of continuous monoculture to wheat (Cook, 1981; Gerlagh, 1968; Shipton, 1975). A similar induction of suppressiveness has been observed over a much shorter time period for Rhizoctonia solani on radishes, alfalfa, and sugar beets (Chet and Baker, 1980; Henis et al., 1978, 1979). In these soils, suppressiveness develops as a result of the build-up of antagonists in response to high pathogen populations produced by the successive growing of susceptible cultivars. Suppression of take-all has been associated with certain fluorescent pseudomonads as well as other bacteria (Cook and Rovira, 1976; Weller and Cook, 1983; Weller et al., 1988), while the suppression of Rhizoctonia solani is attributed to Trichoderma spp. (Chet and Baker, 1980; Henis et al., 1978, 1979; Liu and Baker, 1980).

Most soils known to be suppressive to Fusarium wilt diseases are naturally occurring (Alabouvette, 1986; Alabouvette et al., 1985b; Cook and Baker, 1983; Louvet et al., 1981; Tamietti and Pramotton, 1990). However, a soil suppressive to Fusarium wilt of watermelon that was induced by monoculture of a particular cultivar of watermelon ('Crimson Sweet') has been described and studied (Hopkins et al., 1987; Chapter 3). Few other examples of induced wilt-suppressive soils have been reported. Sneh et al. (1987) reported a soil suppressive to Fusarium oxysporum f. sp. melonis that was induced by continuously cropping to resistant melon varieties for several years. Evidence of a similar induction of suppression in the early 1900's was recounted by Kommedahl et al. (1970), in which long-term monoculture of a cultivar resistant to flax wilt resulted in a marked decline in disease following several years of increases, whereas cropping to susceptible cultivars resulted in complete wilt (100%) every year. Schneider (1984), also observed what may have been an induced suppression to Fusarium wilt of celery where "islands" of healthy celery plants were found in fields otherwise uniformly devastated by wilt. In both of these most recent cases, the organisms responsible were concluded to be isolates of F. oxysporum not pathogenic to the crop plant.

Previously, the population dynamics and chlamydospore germination of F. oxysporum f. sp. niveum, as well as root colonization by F. oxysporum and other microorganism groups, was studied in the 'Crimson Sweet' suppressive monoculture soil and compared with similar conducive soils; some distinct differences were demonstrated among these soils (Chapter 3). Because the induction of suppression in this monoculture soil is linked with cultivation of a particular cultivar, it is valuable to study the effects of such cultivation on different microorganism groups. By comparing the changes in soil and rhizosphere microflora populations directly due to the planting of watermelon and the

differences between cultivars, the important microbiological interactions and their role in suppression can be determined more accurately.

Because previous studies with this suppressive soil, as well as other Fusarium wilt-suppressive soils, have indicated an important role for nonpathogenic F. oxysporum, special emphasis was placed on changes in the populations of F. oxysporum and watermelon root colonization by F. oxysporum. Using four soils representing different suppressive and conducive conditions, the objectives of this study were to evaluate the effect of successive plantings of different watermelon cultivars on the population dynamics and root colonization of F. oxysporum f. sp. niveum, indigenous F. oxysporum, and other general microorganism groups, and the incidence of Fusarium wilt.

Materials and Methods

Soils Used

The four soils used throughout this study to represent different suppressive and conducive conditions to Fusarium wilt of watermelon are from the Central Florida Research and Education Center, Leesburg, FL, and have been described previously (Chapter 3). All are of the Apopka Fine Sand soil series (loamy, siliceous, hyperthermic Grossarenic Paleudults) and have similar physical and chemical characteristics. They differ primarily in their cropping history and the resulting biology. The soil designations and suppressiveness rankings are as follows: 'Crimson Sweet' monoculture soil (CSS) (suppressive); 'Florida Giant' monoculture soil (FGM) (nonsuppressive); Leesburg fallow soil (LFC) (conducive); and microwave-treated CSS soil (CSMW) (conducive).

Successive Plantings of Watermelon and Assay of Fusarium Wilt

An orange-colored mutant strain of the pathogen (FG-OR3), which was comparable to the wild-type, race 1 strain in growth, pathogenicity, and root colonization, was used to distinguish the pathogen from indigenous F. oxysporum in the field soils (Chapter 3). Soils were infested with chlamydospore inoculum of the orange mutant (OM) pathogen as was described previously (Chapter 3).

Infested soil was put in plastic pots in the greenhouse and allowed to equilibrate for approximately 3 weeks. Watermelon seeds of 'Florida Giant,' a cultivar susceptible to Fusarium wilt, or 'Crimson Sweet,' a moderately resistant cultivar associated with soil suppressiveness, were planted in the infested soil (10 seeds/pot, four to six replicate pots/treatment). Plants were maintained at 20-30°C with a maximum light intensity of 500-700 $\mu mol/m^2/s$, and were grown for 4 weeks. Fusarium wilt was assessed by visual inspection of the plants for wilt symptoms several times a week and verified periodically by plating surface-disinfested stem pieces on Komada's (1975) selective medium for F. oxysporum. Wilt was expressed as the percentage of diseased plants over the 4-week period. After the final wilt assessment, plants were harvested, soil and root samples were collected, the soil was mixed thoroughly within the pot, and the pots were replanted with the same cultivar in the same manner. This was continued for four to five successive plantings over a 6-month period. All tests were conducted at least twice.

Population Dynamics and Root Colonization of Fusarium oxysporum

Soil samples of 5 g each were taken from each pot at the time of initial infestation and immediately prior to each successive planting. Populations of F. oxysporum f. sp. niveum (as represented by the OM pathogen) and indigenous F. oxysporum were

determined by serial dilution plating on Komada's (1975) medium as described previously (Chapter 3).

Whole root samples of 3-week-old watermelon plants were gently removed from the pots and rinsed under running water. In some experiments, surface colonization was determined by embedding the roots in molten Komada's (1975) medium as previously described (Chapter 3). Internal colonization was determined by surface-sterilizing roots in 0.5% sodium hypochlorite for one minute, rinsing in sterile water, then plating out as with the others.

Colonies of the OM pathogen, other F. oxysporum, and other fungi were differentiated by color and morphology. In addition, the spatial arrangement of the pathogen in relation to indigenous F. oxysporum and other fungi was observed. The lengths of plated root systems were estimated by a line intersect method (Tennant, 1975) and colonization was expressed as colonies/100 cm root. All experiments used four replications of four to six root systems each and were conducted twice (Chapter 3).

Soil Microorganism Populations

Estimates of general soil microorganism populations were made using standard serial dilution plating procedures as described previously (Chapter 3). Overall populations of aerobic, heterotrophic bacteria were estimated using nutrient agar and actinomycete populations were estimated on alkaline water agar, pH 10.5 (Ho and Ko, 1985). Fluorescent pseudomonad populations were estimated using selective King's medium B with cyclohexamide, penicillin, and novobiocin added (Sands and Rovira, 1970). General fungal populations were estimated on potato dextrose agar with 1 ml tergitol NP-10 and 50 mg chlortetracycline added per liter. All plates were incubated at 26°C. Nutrient agar

and King's medium B plates were incubated 3-4 days, and King' medium B plates were examined under ultraviolet light for colonies producing diffusible fluorescent pigments. Alkaline water agar plates were incubated 7-10 days and total colonies counted. Fungal plates were incubated 5-6 days. Populations were expressed as log colony forming units (log cfu)/g soil and four replications of four plates/treatment were used.

Statistical Analyses

All experiments were analyzed using standard analysis of variance (ANOVA) procedures. Significance was evaluated at $P<0.05$ for all tests and mean separation was accomplished using Duncan's multiple range test. Experimental designs for most tests were variations on a randomized complete block. Most simple analyses and individual factor comparisons were conducted using a one way ANOVA. Overall results of two or more factor experiments used a two way ANOVA. Linear regression or correlation analysis was used on successive planting tests where appropriate. All data expressed as percentages were arcsin-transformed ($\sin^{-1}\sqrt{x}$) before analysis. All computations were made using Statgraphics (STSC Inc.) statistical computer program on a personal computer.

Results

Population Dynamics of Fusarium oxysporum and Fusarium Wilt

Five successive plantings of watermelon cultivars 'Florida Giant' and 'Crimson Sweet' resulted in distinct differences in the population of F. oxysporum f. sp. niveum among the four soils (Figure 4-1). After the equilibration period (3 weeks) and at the time of the first planting, pathogen populations in the suppressive (CSS) soil were lower than

in the two conducive soils (LFC and CSMW) for both cultivars, but were not different than those in nonsuppressive monoculture (FGM) soil. This increase in the pathogen populations of the conducive soils before planting was similar to that observed previously in unplanted soils (Chapter 3). Significant differences were noted in OM pathogen populations among the conducive soils and the monoculture soils beginning with the second planting of 'Florida Giant' and at each subsequent planting. With cultivar 'Crimson Sweet,' pathogen populations in the two monoculture soils were also lower than in the conducive soils at all plantings except the fourth.

Comparisons within each soil (ANOVA, $P<0.05$) determined that successive plantings of either cultivar 'Florida Giant' or 'Crimson Sweet' had no effect on pathogen populations in either monoculture (CSS or FGM) soil throughout the study period. In the conducive soils, plantings of 'Florida Giant' resulted in increasing pathogen populations with each successive planting (ANOVA, linear regression analysis, $P<0.05$, $b=3.31 \pm .41$ and $3.05 \pm .29$ for CSMW and LFC soil, respectively). With planting to 'Crimson Sweet,' however, after the initial population increases in the raw soils, pathogen populations levelled off and stabilized, showing no further increases throughout the remaining successive plantings in the conducive soils.

Thus, there were also differences observed in comparisons between OM pathogen populations in the conducive soils planted to 'Florida Giant' versus 'Crimson Sweet;' CSMW and LFC soil planted to 'Florida Giant' averaged 1956 and 1613 cfu/g soil by the fifth planting, while CSMW and LFC soil planted to 'Crimson Sweet' averaged 881 and 731 cfu/g soil, respectively. Populations of the pathogen were more stable in the monoculture soils; by the fifth planting, CSS and FGM soils had averages of 206 and 250

cfu/g soil, respectively, when planted to 'Crimson Sweet' and 375 and 393 cfu/g soil with 'Florida Giant.'

Estimates of soil populations of indigenous F. oxysporum (all F. oxysporum other than the OM pathogen) also demonstrated differences with successive plantings of 'Florida Giant' and 'Crimson Sweet' (Figure 4-2). When planted to 'Florida Giant,' populations of F. oxysporum fluctuated widely in each soil, but showed no consistent change with successive plantings and stayed within the normal range associated with that soil. Soil populations of F. oxysporum also fluctuated when planted to 'Crimson Sweet,' but showed a significant trend of increase with successive plantings in LFC, FGM, and CSS soils (ANOVA, linear regression analysis, $P<0.05$, $b=3.18 \pm .48$, $2.23 \pm .67$, 3.88 ± 1.67, for LFC, FGM, and CSS soils respectively). By the fifth planting of 'Crimson Sweet,' populations averaged 2630, 2220, and 1593 cfu/g soil in CSS, FGM, and LFC soils, respectively, as compared to 1098, 1424, and 212 cfu/g soil, respectively, at the beginning of the experiment. The highest populations were in CSS and FGM soil planted to 'Crimson Sweet,' with populations in CSS soil greater than all others at the second and third planting. The population of F. oxysporum in CSS soil also was greater than in LFC soil throughout the experiment. When planted to 'Florida Giant,' the population of F. oxysporum in CSS soil was not different than that in FGM soil at any planting after initial infestation. There was virtually no detectable population of indigenous F. oxysporum in CSMW soil throughout most of the experiment due to the microwave treatment this soil received. The low levels of F. oxysporum observed in CSMW soil at plantings four and five represent limited recolonization.

Fusarium wilt levels in soils planted to 'Florida Giant' were high throughout all plantings in conducive soils and tended to increase with the first few successive plantings

in the monoculture soils (Figure 4-3). Suppression of Fusarium wilt in CSS soil, which was evident at the first planting (significantly lower wilt than all other soils), was no longer present by the third planting or thereafter. No differences in level of wilt between CSS and the other soils were observed after the third planting and all soils showed disease levels of 70% and greater. Although in this test the disease level in FGM soil did not continue to increase through the fourth and fifth planting of 'Florida Giant,' a repeat of this experiment as well as a similar additional experiment showed increasing wilt through the fourth or fifth planting (Appendix A; Figure A-1 and Appendix B; Figures B-1 to B-3). In contrast, Fusarium wilt remained low (20-27% wilt) throughout the five successive plantings of 'Crimson Sweet' in CSS soil and also did not increase in FGM soil, although initial levels were higher (34-42%) than those in CSS soil (Figure 4-3). In LFC and CSMW soils, wilt levels were high in the first planting, but did not change dramatically with successive plantings (46-66% and 65-83% for LFC and CSMW soils, respectively). Differences in wilt suppression among the soils were evident through each successive planting, with CSS soil maintaining lower disease levels than those in LFC or CSMW soils. A repeat of this experiment showed similar results regarding F. oxysporum f. sp. niveum, indigenous F. oxysporum, and wilt levels, with the exceptions already noted and that of showing declining wilt levels in LFC soil successively planted to 'Crimson Sweet' (Appendix B; Figures B-2 and B-5).

Root Colonization by Fusarium oxysporum

Root surface colonization measured after four successive plantings of 'Crimson Sweet' and 'Florida Giant' revealed differences among the soils (Table 4-1). Colonization of 'Crimson Sweet' by F. oxysporum f. sp. niveum was lowest in the two monoculture

soils, but was not different between CSS and FGM soil. Root colonization by the pathogen was greater in LFC and CSMW soils than in both monoculture soils, with CSMW soil demonstrating the highest level of colonization. Colonization by indigenous F. oxysporum was several times that by F. oxysporum f. sp. niveum in both CSS and FGM soils, both of which had greater levels of colonization by F. oxysporum than those in LFC and CSMW soils. Colonization by F. oxysporum was lowest in CSMW soil, but did show substantial root colonization due to recolonization of the soil over time after microwave treatment. The ratio of colonization by the pathogen to colonization by indigenous F. oxysporum also was similar in CSS and FGM soils and was less than in the two conducive soils. Root surface colonization by the pathogen was correlated (r=.71) to soil population levels of the pathogen at the time of root sampling. Colonization by F. oxysporum also was correlated (r=.69) with its respective soil populations as was observed previously (Chapter 3). Colonization by the pathogen was negatively correlated (r=-.79) with colonization by F. oxysporum. Wilt was lower in CSS than in LFC and CSMW soils with cultivar 'Crimson Sweet.' Wilt levels also increased and were correlated (r=.68) with soil populations of the pathogen.

With planting to cultivar 'Florida Giant,' slightly higher levels of colonization by F. oxysporum f. sp. niveum were observed for all soils, and differences between the monoculture soils (CSS and FGM) and the conducive soils were similar to those observed with cultivar 'Crimson Sweet' (Table 4-1). Colonization by indigenous F. oxysporum was slightly lower in FGM, LFC, and CSMW soil than with cultivar 'Crimson Sweet,' but followed the same pattern of differentiation as observed previously, as did the colonization ratio of F. oxysporum f. sp. niveum/F. oxysporum. However, wilt was high in all soils with no significant differences among them. Wilt, then, was not directly related to the level of

colonization by the pathogen or indigenous F. oxysporum. Soil populations of the pathogen at the time of sampling were lower in the monoculture soils than in conducive soils, and populations of F. oxysporum were highest in monoculture soils planted to 'Crimson Sweet.' Significant differences were also observed when comparisons were made between cultivars (ANOVA, Duncan's, $P<0.05$) with colonization by F. oxysporum f. sp. niveum higher in FGM and LFC soil when successively planted to 'Florida Giant' versus 'Crimson Sweet.' Similar results were observed in a repeat of this experiment (Appendix A; Table A-9).

Internal root colonization by F. oxysporum f. sp. niveum, measured using surface-disinfested roots, was not different between CSS and FGM soil, although both monoculture soils had lower colonization by the pathogen than that in LFC soil (Table 4-2). Colonization by indigenous F. oxysporum was similar between all three soils. The ratio of colonization by the pathogen/F. oxysporum was similar in the monoculture soils, but lower than in LFC soil. Wilt levels were again significantly different among the three soils; wilt was low in CSS soil, moderate in FGM soil, and severe in LFC soil.

Soil Microorganism Populations

Successive plantings of cultivar 'Crimson Sweet' in CSS, FGM, and CSMW soil resulted in increases in populations of bacteria, actinomycetes, fluorescent pseudomonads, and other pseudomonads compared to unplanted soil (Figure 4-4). Increases in populations were observed in LFC soil for actinomycetes, fluorescent pseudomonads, and other pseudomonads, but not for overall bacteria. In FGM soil, population increases following planting to 'Crimson Sweet' also were greater than after planting to 'Florida Giant' for all microorganism groups except fungi. Populations of

actinomycetes and fluorescent pseudomonads also were greater in CSS and CSMW soils when planted to 'Crimson Sweet' than to 'Florida Giant.' Overall, in all soils, planting watermelon ('Florida Giant' or 'Crimson Sweet') caused increases in the general prokaryotic microorganism populations as measured in this study. Planting watermelon had no effect on overall fungal populations in any soil, except LFC, in which overall fungal populations decreased when successively planted to 'Crimson Sweet' or 'Florida Giant.' Fungal populations also were lower when planted to 'Crimson Sweet' than to 'Florida Giant' in this soil. LFC soil had the highest overall fungal populations and lowest bacterial populations initially.

Discussion

In this study, cultivation of watermelon, as well as the particular cultivar planted, had significant effects on the populations of F. oxysporum pathogenic and not pathogenic to watermelon. When planted to the susceptible cultivar 'Florida Giant,' pathogen populations tended to increase with successive planting, while populations of indigenous F. oxysporum did not change significantly. When planted to 'Crimson Sweet,' pathogen populations in all soils did not differ substantially from those observed in unplanted soils (Chapter 3), regardless of the number of plantings. Populations of indigenous F. oxysporum, however, tended to increase overall with successive planting to 'Crimson Sweet' in the field soils. Thus, 'Crimson Sweet' appeared to selectively favor the growth of nonpathogens over pathogens, whereas 'Florida Giant' tended to promote pathogen development over nonpathogens. Several studies have demonstrated that susceptible crops increase pathogen populations, whereas nonhost, or resistant hosts, even when

Figure 4-1. Population dynamics of Fusarium oxysporum f. sp. niveum (as represented by an orange-colored mutant pathogen) in four soils with successive plantings of two different watermelon cultivars (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment). A) Watermelon cultivar 'Florida Giant' (susceptible to Fusarium wilt); B) watermelon cultivar 'Crimson Sweet' (moderately resistant to Fusarium wilt and inducer of soil suppressiveness). Population estimates were made at the time of planting for each planting number. Values within each planting for each cultivar topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

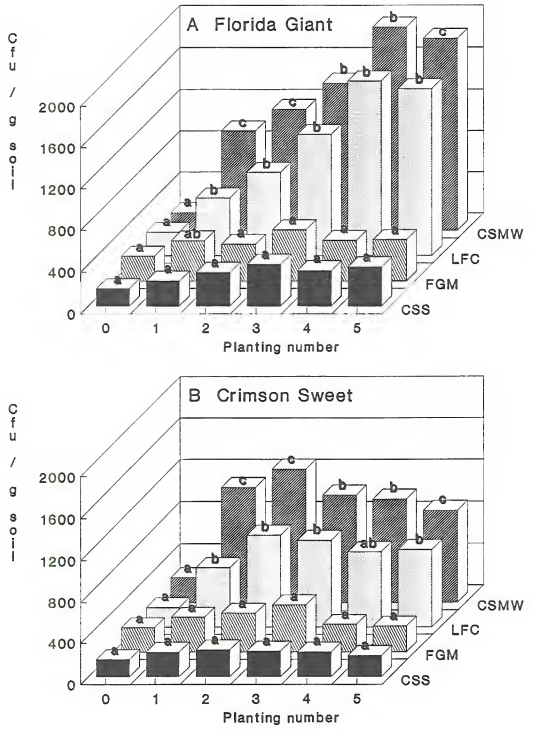

A Florida Giant
B Crimson Sweet
Cfu / g soil
2000
1600
1200
800
400
0
0 1 2 3 4 5
Planting number
CSMW
LFC
FGM
CSS

Figure 4-2. Population dynamics of indigenous Fusarium oxysporum in four soils with successive plantings of two different watermelon cultivars (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment). A) Watermelon cultivar 'Florida Giant' (susceptible to Fusarium wilt); B) watermelon cultivar 'Crimson Sweet' (moderately resistant to Fusarium wilt and inducer of soil suppressiveness). Population estimates were made at the time of planting for each planting number. Values within each planting for each cultivar topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

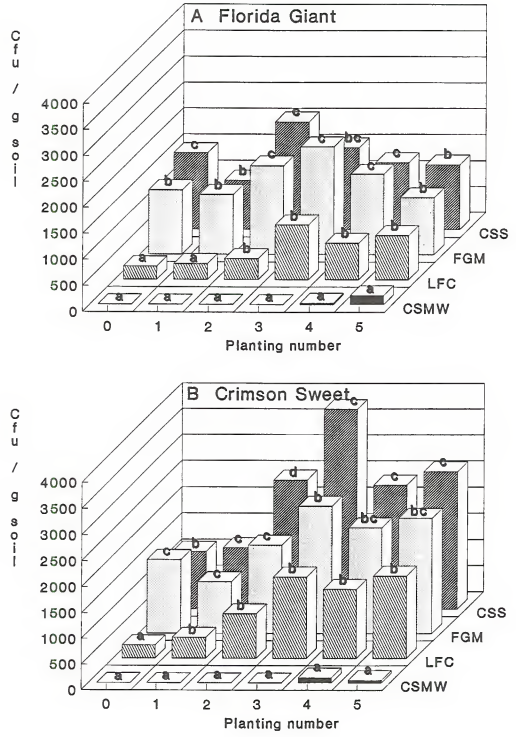
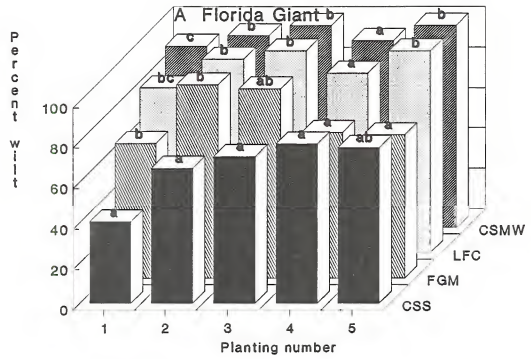

A Florida Giant
B Crimson Sweet
Cfu / g soil
4000
3500
3000
2500
2000
1500
1000
500
0
0 1 2 3 4 5
Planting number
CSS
FGM
LFC
CSMW

Figure 4-3. Fusarium wilt in four soils with successive plantings of two different watermelon cultivars (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment). A) Watermelon cultivar 'Florida Giant' (susceptible to Fusarium wilt); B) watermelon cultivar 'Crimson Sweet' (moderately resistant to Fusarium wilt and inducer of soil suppressiveness). Values within each planting topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.


A Florida Giant
Percent wilt
100
80
60
40
20
0
1
2
3
4
5
Planting number
CSMW
LFC
FGM
CSS

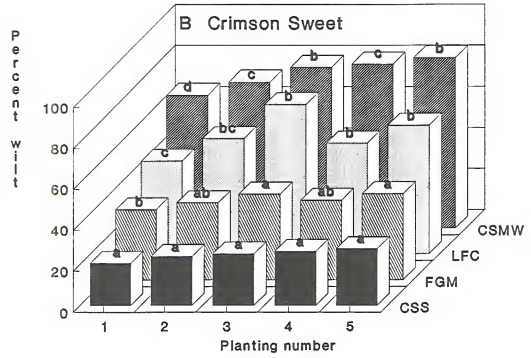

B Crimson Sweet
Percent wilt
100
80
60
40
20
0
1
2
3
4
5
Planting number
CSMW
LFC
FGM
CSS

Table 4-1. Surface colonization of roots of two watermelon cultivars by Fusarium oxysporum in relation to soil populations and disease incidence in four soils after four successive watermelon plantings.

Soil type[a]	Colonies per 100 cm root[b]		Colonization ratio[c]	% Wilt	Soil populations[d] (cfu/g)	
	OM pathogen	F. oxysporum			OM pathogen	F. oxysporum
'Crimson Sweet'						
CSS	10.6a[e]	79.8a	0.14a	26.3a	240a	2375a
FGM	18.2a	78.7a	0.23a	38.7ab	270a	2038ab
LFC	29.0b	50.3b	0.78b	53.7bc	730b	1338b
CSMW	67.6c	30.8c	2.66c	80.0c	990b	133c
'Florida Giant'						
CSS	21.8a	97.5a	0.24a	78.8a	340a	1294a
FGM	38.4ab	68.3a	0.53a	72.0a	387a	1550a
LFC	49.6b	36.2b	1.67b	62.0a	1690b	700b
CSMW	71.8c	19.8c	3.63c	92.5a	1960b	25c

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive); LFC=Leesburg fallow conducive soil; CSMW=microwave-treated, 'Crimson Sweet' soil (conducive).

[b] Colonization of roots by the orange mutant (OM) strain of F. oxysporum f. sp. niveum and all F. oxysporum other than the OM pathogen was determined by washing the roots of 3-week-old plants and embedding them intact in Komada's (1975) medium. Four replications of four to six roots each were used. Root length was estimated by the line-intersect method (Tennant, 1975).

[c] The colonization ratio represents the mean of the colonization by the OM pathogen divided by the colonization by other F. oxysporum calculated for each sample.

[d] Soil populations of the OM pathogen and other F. oxysporum were determined by dilution-plating at the time of root colonization measurements. Initial inoculum of OM pathogen was approximately 200 cfu/g soil.

[e] Means within columns for each cultivar followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Table 4-2. Internal colonization of 'Crimson Sweet' watermelon roots by Fusarium oxysporum in soils suppressive and conducive to Fusarium wilt following four successive plantings.

Soil type[a]	Colonies per 100 cm root[b] OM pathogen	F. oxysporum	Colonization ratio[c]	% Wilt
CSS	1.17a[d]	1.59a	1.31a	9.9a
FGM	4.68a	2.37ab	1.44a	35.1b
LFC	15.70b	2.55a	9.13b	61.4c

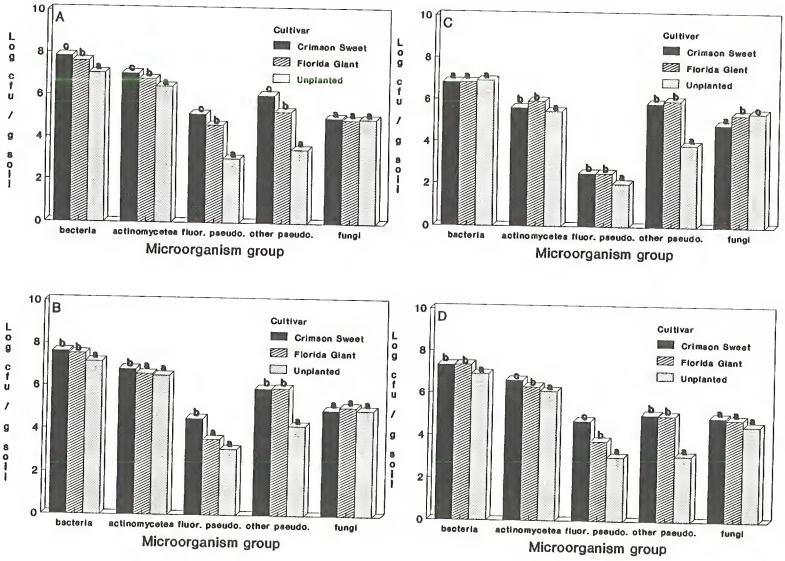
[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive); LFC=Leesburg fallow soil (conducive); CSMW=microwave-treated, 'Crimson Sweet' soil (conducive).

[b] Colonization of roots by the orange mutant (OM) strain of F. oxysporum f. sp. niveum and all F. oxysporum other than the OM pathogen was determined by washing the roots of 3-week-old plants and embedding them intact in Komada's (1975) medium. Four replications of four to six roots each were used. Root length was estimated by the line-intersect method (Tennant, 1975).

[c] The colonization ratio represents the mean of the colonization by the OM pathogen divided by the colonization by other F. oxysporum calculated for each sample.

[d] Means in columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Figure 4-4. Populations of bacteria, actinomycetes, fluorescent pseudomonads, other pseudomonads, and fungi in four soils after four successive plantings of watermelon cultivars 'Florida Giant' and 'Crimson Sweet.' A) FGM (nonsuppressive, monoculture) soil; B) CSS (suppressive, monoculture) soil; C) LFC (fallow, conducive) soil; D) CSMW soil (suppressive soil rendered conducive by microwave treatment). Values within each microorganism group topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.


A
B
C
D
Cultivar
Crimson Sweet
Florida Giant
Unplanted
Log cfu / g soil
bacteria
actinomycetes
fluor. pseudo.
other pseudo.
fungi
Microorganism group

colonized by the pathogen, do not result in pathogen population increases (Banihashemi and deZeeuw, 1975; Katan et al., 1983; Reyes and Mitchell, 1962; Wensley and McKeen, 1963). This may explain why suppressive soil maintains low pathogen populations, yet no direct evidence of pathogen suppression could be determined (Chapter 3). The low pathogen to nonpathogen balance may be maintained by a selection for certain organisms.

Planting watermelon also had significant effects on other microorganism populations, with overall populations of bacteria, actinomycetes, and pseudomonads increasing after a number of successive plantings. This general increase due to planting is not surprising, however, because plant roots provide much nutrition in the form of exudates to relatively low-nutrient soils, thus enabling more organisms of all kinds to survive (Foster and Bowen, 1982). There also were differences between cultivars, with planting to 'Crimson Sweet' resulting in overall greater populations for most prokaryote groups than planting to 'Florida Giant.'

These alterations in the populations of F. oxysporum and bacteria suggest the promotion of differences in rhizosphere microflora populations caused by cultivar differences. Although it is readily acknowledged that rhizosphere microflora are influenced by the host, there is very little known regarding the degree and level of control plant genotype has on the composition of rhizosphere microflora. Work done by Atkinson and co-workers (1973; Neal et al., 1973) demonstrated that host genotype of spring wheat had significant and specific effects on the composition of its rhizosphere microflora. More recently, Bourbos and Skoudridakis (1987) found that a resistant tomato cultivar specifically promoted the growth of various fungi antagonistic to plant pathogens in the rhizosphere, whereas a susceptible cultivar did not. There also have been other reports

on various effects of varietal differences on rhizosphere microflora (Buxton, 1975; Mall, 1979; Subba-Rao and Bailey, 1961). The situation observed in this study may be another case where cultivar differences are sufficient to substantially change the rhizosphere microflora, either by enhancing microorganism populations in general or by selectively favoring certain groups or strains of organisms. The mechanism by which this stimulation takes place is not known, but presumably results from differences in the quantity and composition of root exudates.

Additional tests with successive plantings of other watermelon cultivars, 'Calhoun Gray,' a highly resistant cultivar, and 'Charleston Gray,' a moderately resistant cultivar, also suggested that the level of cultivar resistance affects microflora populations (Appendix B; Figures B-7 to B-10). 'Calhoun Gray' and 'Crimson Sweet' generally showed somewhat higher total populations of bacteria, actinomycetes, and pseudomonads than the less resistant cultivars 'Charleston Gray' and 'Florida Giant.' The population levels observed corresponded roughly with levels of resistance in the field. Neither of these two additional cultivars promoted detectable soil suppressiveness, however. Several other cultivars classified as having equal or greater levels of resistance than 'Crimson Sweet' also did not induce suppression in the field (Hopkins and Elmstrom, 1984; Hopkins et al., 1987). Thus, there is evidently more to this suppression than cultivar resistance and a general stimulation of microorganism activity.

Although most bacterial groups were affected by planting to watermelon, in all but LFC soil, there was no noticeable effect on total fungal populations. However, there was a significant effect on populations of F. oxysporum (either the OM pathogen or indigenous F. oxysporum or both) in all soils. This illustrates that F. oxysporum is only a small component of the total fungal flora (F. oxysporum populations averaged 10^2-10^3

versus 10^5 cfu of total fungi/g soil), and fluctuations of one species may not be reflected in the total population estimates. This general relationship of F. oxysporum compared to overall fungal populations in the rhizosphere is similar to that reported by others (Banihashemi and deZeeuw, 1975; Gordon et al., 1989). The decrease in fungal populations in LFC soil is presumably a result of the increase in bacterial numbers. It may be that this conducive soil is slowly converting to the microbiology that characterizes suppressive soil through the planting of watermelon. In some of the wilt assays, this possible conversion toward suppression could be seen as a reduction in wilt in LFC soil after a number of successive plantings of 'Crimson Sweet' (Appendix B; Figures B-2 and B-5). Although overall fungal populations decreased in this soil, the reduction of wilt may have been related to changes within the population structure of F. oxysporum; this move towards suppression corresponds with increases observed in indigenous populations of F. oxysporum versus the lack of an increase in the OM pathogen population with successive plantings of 'Crimson Sweet.' Although only general numbers of organisms were assayed in this study and no information on the possible changes in the composition or diversity of species is available, changes in population numbers were evidence of some effect on the microflora. The significant effect of planting on populations of F. oxysporum in these soils suggests a specific relationship between F. oxysporum and planting to watermelon that was not reflected in total fungal populations.

Differences in level of disease also were observed between planting to the two cultivars in these soils. Wilt, in general, tended to increase with successive plantings of 'Florida Giant,' but not with plantings of 'Crimson Sweet.' Although direct comparison between these cultivars is complicated by their difference in resistance level, cultivar 'Crimson Sweet' is classified as moderately resistant and 'Florida Giant' is considered

susceptible (Elmstrom and Hopkins, 1981; Hopkins and Elmstrom, 1984), this difference in resistance does not totally explain the differences in suppressiveness observed. Although 'Florida Giant' does show greater levels of wilt than 'Crimson Sweet' in the same soils, initial differences in suppressiveness between soils were apparent with both cultivars. However, after just two plantings of 'Florida Giant,' all indications of differences in disease suppression between the soils were removed. This was not due merely to an increase in pathogen population, because an increase in disease was noted in CSS and FGM soils even when OM pathogen populations did not substantially increase. Suppressiveness was maintained with 'Crimson Sweet' and eliminated with 'Florida Giant.' Sneh et al. (1987) also found that planting to susceptible cultivars would nullify a similarly induced suppression after as little as two plantings.

Root colonization of 'Crimson Sweet' by F. oxysporum f. sp. niveum did not change in CSS, FGM, and LFC soil after successive plantings, but did substantially increase in CSMW soil compared to initial levels observed previously (Chapter 3). Root colonization by indigenous F. oxysporum was greater in all soils after successive plantings than observed in initial plantings (Chapter 3), and colonization in the monoculture soils was significantly greater than in the conducive soils. Colonization by F. oxysporum f. sp. niveum was negatively correlated (r=.71) with colonization by F. oxysporum when planted to 'Crimson Sweet,' but not with 'Florida Giant.' Even after a number of successive watermelon plantings, however, there was no difference observed in colonization by F. oxysporum f. sp. niveum, indigenous F. oxysporum or their ratio between the two monoculture soils (CSS and FGM), although large differences were often observed between these and LFC soil. Thus, despite differences in population dynamics of F. oxysporum f. sp. niveum and indigenous F. oxysporum due to planting to

watermelon, root colonization by the OM pathogen and indigenous F. oxysporum (whether surface or internal) was similar in both suppressive and nonsuppressive monoculture soils, and was not consistently related to wilt. As discussed previously (Chapter 3), this may suggest that specific strains of nonpathogenic F. oxysporum rather than general populations may be involved in suppression. These specific antagonist strains may be more abundant in CSS than FGM soil, but can not be distinguished by colonization data. Thus, if nonpathogenic strains are involved in suppression, it does not appear that the ability to colonize effectively is a distinguishing trait; nonpathogens in FGM soil were able to colonize as well as those in CSS soil, but did not suppress disease. However, the problem associated with indigenous pathogens being included in indigenous F. oxysporum counts in this study has been recognized, and does limit the conclusions that can be made regarding nonpathogenic populations of F. oxysporum (Chapter 3).

Differences in microorganism populations were previously observed between suppressive and conducive soils (Chapter 3). In this study, changes in the populations of F. oxysporum and various bacteria were directly related to plantings of watermelon, and particularly cultivar 'Crimson Sweet.' From the observed changes in pathogenic and nonpathogenic populations of F. oxysporum, it is evident that planting watermelon has a profound effect on populations of F. oxysporum, their development, and the distribution of pathogens versus nonpathogens. Because these specific changes in the dynamics of F. oxysporum are due to the cultivar that promotes suppressiveness and F. oxysporum has often been implicated in the suppression of Fusarium wilt in other soils, it suggests that the relationship between pathogenic and nonpathogenic F. oxysporum is involved in this suppressive response. However, it is also quite likely that other organisms, such

as fluorescent pseudomonads or other bacteria may be involved, because these organisms also showed significant population changes due to planting.

The effect of monoculture on soilborne plant pathogens and disease varies considerably with different pathosystems and the conditions within them. Traditionally, monoculture has been associated with increases in pathogen populations and disease, but more recently, the benefits of monoculture in creating a natural form of biological control in certain pathosystems have been recognized (Cook and Baker, 1983; Shipton, 1977). However, despite these benefits, monoculture can also cause problems related to soil fertility, chemistry, and physiology.

The response of F. oxysporum and Fusarium wilt to monoculture, as represented by numerous different crops and formae speciales, has covered virtually every conceivable possibility, including irreversible increases in disease, limited development of disease, failure of the pathogen to establish, and disease development followed by decline (Cook and Baker, 1983; Hopkins and Elmstrom, 1984; Hopkins et al., 1987; Schneider, 1982; Shipton, 1977; Sneh et al., 1987). These differences have been attributed to differences between formae speciales (Shipton, 1977). However, when the responses are broken down by susceptible versus selected resistant cultivars and suppressiveness related to physical soil factors eliminated, a consistent pattern emerges. Monoculture of susceptible cultivars have resulted in irreversible increases in disease while certain resistant cultivars have resulted in disease decline or soil suppressiveness.

This response apparently differs from other pathosystems involving induced suppression, such as seen with take-all decline (Cook, 1981; Shipton, 1975) and Rhizoctonia solani (Chet and Baker, 1981; Henis et al., 1978, 1979), where susceptible cultivars are involved and their is no apparent interaction with host resistance. This raises

many interesting questions regarding the concepts and mechanisms of resistance to Fusarium wilt. Although the successive planting experiments in this study are not directly comparable to the conditions of monoculture in the field, these tests indicated possible effects as a result of monoculture. Cultivar 'Crimson Sweet,' which is responsible for the induction of suppression, also caused many changes in the soil and root microbiology of the these soils, presumably through the effect of differences in host genotype on the rhizosphere microflora. A resistance mechanism such as this, of the host enhancing the growth and establishment of organisms antagonistic to plant pathogens, may represent a new direction in biological control. Development of the biological control potential on both sides of this system through isolation, identification, and utilization of specific effective antagonistic organisms as well as the incorporation of the inherent genetic ability of the plant to enhance the development of such organisms, may enable an integrated biological control that may be effective in overcoming difficulties often encountered with the introduction of antagonist organisms.

CHAPTER 5
THE ROLES OF INDIGENOUS FUSARIUM OXYSPORUM AND VARIOUS OTHER MICROORGANISMS IN A SOIL SUPPRESSIVE TO FUSARIUM WILT OF WATERMELON

Introduction

Previous studies with the 'Crimson Sweet' suppressive monoculture soil have focused on the general ecological characteristics of the pathogen in relation to other indigenous microorganism groups in suppressive versus conducive soil, and the effect of watermelon cultivation on these characteristics (Chapters 3 and 4). This work suggested that specific antagonistic organisms, rather than general population levels of microorganisms, were responsible for suppressiveness. Therefore, in this study potential antagonists were isolated from suppressive soil and evaluated for their possible roles in the suppressive response. Since previous work also indicated that indigenous strains of Fusarium oxysporum were likely involved in this suppression, special emphasis was placed on the interaction of the pathogen with isolates of F. oxysporum not pathogenic to watermelon. Because the pathogen is biologically active within the rhizosphere and must invade the root before disease can result, this study focused on rhizosphere- and rhizoplane-colonizing organisms.

The objectives of this study were to isolate a wide variety of potentially antagonistic organisms from the roots of watermelon plants growing in suppressive and nonsuppressive soil, to screen them for their ability to restore suppressiveness and reduce disease in microwave-treated and field soil, and, finally, to evaluate which

characteristics of successful antagonists contribute to their effectiveness and mechanism of action. A preliminary report of portions of this work has been published (Larkin et al., 1990).

Materials and Methods

Soil Infestation and Assay of Fusarium Wilt

The four soils used in this study to represent different suppressive and conducive conditions were described previously (Chapter 3) and consisted of the suppressive, 'Crimson Sweet' (CSS) monoculture soil; a nonsuppressive, 'Florida Giant' monoculture (FGM) soil; a conducive field soil (LFC) that had not been planted to watermelon in eight years; and a suppressive soil that had been rendered conducive (CSMW) by microwave treatment of 90 s/kg soil (700 watts, 2450 MHz) at a soil matric potential of -0.01 MPa.

An orange-colored mutant strain of the pathogen (OM), which was comparable to the parental wild-type of a race 1 strain in growth, pathogenicity, and root colonization, was used to distinguish the pathogen from indigenous F. oxysporum in the field soils (Chapter 3). Soils were infested with chlamydospore inoculum of the OM pathogen (200-400 cfu/g soil) as described previously (Chapter 3). Watermelon seeds were planted in pots of the infested soil (four to six plants in four to six replicate pots depending on the individual experiment) in the greenhouse. Plants were maintained at 20-30°C with a maximum light intensity of 500-700 $\mu mol/m^2/s$ and were grown for 4 weeks. Fusarium wilt was assessed by visual inspection of the plants for wilt symptoms several times a week and verified periodically by plating surface-sterilized stem pieces on Komada's (1975) selective medium for F. oxysporum. Wilt was expressed as the percentage of diseased plants over the 4-week period.

Isolation of Potential Antagonists

Roots from 'Crimson Sweet' watermelon plants grown in CSS and FGM soils that had been microwave-treated for 0, 30, or 60 s/kg soil were used for rhizosphere and rhizoplane organism dilution platings as described previously (Chapter 3). Bacterial isolates were obtained from nutrient agar and 1/10 strength tryptic soy agar plates. Actinomycetes were selected for on alkaline water agar at pH 10.5 (Ho and Ko, 1980). Pseudomonads were isolated on a selective King's medium B containing penicillin, cyclohexamide, and novobiocin (Sands and Rovira, 1970). Plates of King's medium B were examined under ultraviolet light for colonies producing diffusible fluorescent pigments to identify fluorescent strains. Fungal organisms were isolated on potato dextrose agar containing 1 ml tergitol NP-10 and 50 mg chlortetracycline/l medium. Fusarium oxysporum was isolated from surface-disinfested roots as well as rhizosphere and rhizoplane dilutions using Komada's (1975) medium. Plates were prepared (minimum of four replicate plates/treatment/media/dilution) and incubated as described previously (Chapter 3). Isolates to be tested as potential antagonists were selected randomly from the agar plates such that representative organisms from all colony and morphology types present were collected in approximate proportion to their abundance on the plates. Organisms other than F. oxysporum and fluorescent pseudomonads were not identified, but were assigned isolate numbers based on the treatment and medium from which they were isolated. An additional 40 isolates of F. oxysporum that had been collected from bulk CSS soil samples in a previous study (Chapter 2) also were tested as antagonists. Included in these were four isolates that were weakly pathogenic on watermelon (VCG 0081, race 1).

Screening Isolates in Microwave-Treated and Field Soils

All collected isolates were screened individually for their ability to restore suppressiveness and reduce disease when added to microwave-treated suppressive soil infested with chlamydospore inoculum of the pathogen at 200-400 cfu/g soil. This test was used as an initial screening stage to identify organisms with any antagonistic potential toward Fusarium wilt. This test also served to reduce the total number of organisms that would need to be screened in field soil tests, since only isolates that indicated antagonistic activity in microwave-treated soil would be tested further.

Fungal isolates were grown for 5 days on potato dextrose agar at 26°C, conidial suspensions of 1-2 x 10^6/ml were prepared in sterile water, and 10 ml of each suspension were mixed into 1 kg infested, microwave-treated, suppressive soil. Bacterial isolates were grown on nutrient agar for 48 hr, suspended in sterile water, and adjusted to an optical absorption of about 0.5 (read at 600 nm), which produced cell counts in the range of 10^7-10^9/ml. A 10-ml aliquot of this suspension was mixed into 1 kg soil. Final concentrations of the organisms in soil were 1-2 x 10^4 cfu/g soil for F. oxysporum and most other fungi and 10^5-10^7 cfu/g soil for most bacteria. Seeds of watermelon cultivar 'Crimson Sweet' were planted (five/pot, five pots/treatment) and wilt was assessed over 4 weeks; data were compared with pathogen-infested, CSS and CSMW control soils for percent wilt, percent suppression (relative to pathogen-infested, CSS soil), and percent reduction in disease (relative to pathogen-infested, CSMW soil). Analysis of variance and mean separation using Duncan's multiple range test ($P<0.05$) were conducted on arcsin-transformed ($\sin^{-1}\sqrt{x}$) percentage data for all tests.

All isolates demonstrating a significant reduction of disease in microwave-treated soil were tested for their ability to reduce disease in a conducive field soil (LFC) infested

with chlamydopspore inoculum of the pathogen at 200-400 cfu/g soil. Isolates were cultured in the same way as for the microwave-treated soil tests, except cell suspensions were washed twice in sterile water before being mixed into pathogen-infested soil. All field soil screening tests were conducted at least twice.

Characteristics of Successful Antagonists

Internal colonization of watermelon roots by the OM pathogen and other isolates of F. oxysporum was determined for selected treatments at the conclusion of the screening trials in both microwave-treated and field soil tests by embedding surface-disinfested root systems in Komada's (1975) medium according to methods described previously (Chapter 3). To evaluate the relationship of root colonization by the pathogen and other isolates of F. oxysporum to suppression of disease, colonization by the OM pathogen and other F. oxysporum was compared with the ability of each isolate to reduce disease.

Vegetative compatibility was analyzed among a number of the isolates of F. oxysporum, including many that were successful and many that were not successful in reducing disease. Nitrate-nonutilizing mutants of selected isolates were produced on a chlorate-amended medium and tested for vegetative compatibility as described previously (Chapter 2). Pairings were made with tester isolates for the established vegetative compatibility groups (VCGs) within F. oxysporum f. sp. niveum (VCG 0080, 0081, and 0082; Chapter 2) as well as all combinations among the test isolates.

Effective antagonists were characterized for similarities which could be related to their effectiveness or mechanism of action. In addition to colonization, effective and noneffective isolates were compared by their source of isolation and the conditions under

which they were isolated to determine if the type of sample or the treatment received had any effect on the number or percentage of effective isolates recovered.

Induced Systemic Resistance

To test the possibility of induced systemic resistance as a mechanism of suppression and to separate this response from competition at or on the roots, a split-root technique was employed. This enabled the physical separation of the pathogen from the antagonists and indigenous microorganisms in suppressive soil. Watermelon seeds were germinated in moist paper towels; upon emergence of the developing root, the root tip was excised, and the root was allowed to grow for 2-3 more days. This resulted in a proliferation of rootlets near the point of excision. A number of rootlets could then be separated into two similar halves for transplanting to a dual-pot system consisting of two square plastic pots (6.5 cm x 9 cm-high) taped together. One pot of each pair contained CSMW, CSS, LFC, or CSMW soil with an antagonist added (four replications of five plants for each treatment). The other pot of each treatment contained 100 g of CSMW soil with the OM pathogen added at 400 cfu/g soil in the bottom half of the pot plus 150 g of CSMW soil without the pathogen in the top half of the pot (Figure 5-1). In this way, roots could grow 2-3 days through suppressive or antagonist-infested soil before being challenged through colonization by the pathogen in the adjoining pot. The pots were initially filled only 2/3 full and the transplant was placed on the rim between the two pots with the roots evenly split between them. The pots were then filled to the top with the appropriate soil and tamped down around the roots. For the antagonist treatment, one half of the root was dipped in a conidial suspension (10^6 cfu/ml) of a nonpathogenic isolate of F. oxysporum before being transplanted into CSMW soil. Plants were allowed

to grow 5 weeks. Root systems and stem sections of wilted plants were surface-disinfested and plated on Komada's (1975) medium. Root systems and stem sections of all plants were plated at the conclusion of the experiment. This study was conducted twice.

Results

Isolation and Screening of Potential Antagonists

A total of 140 isolates of F. oxysporum and 255 isolates of miscellaneous bacteria, actinomycetes, and fungi were collected from the roots of 'Crimson Sweet' watermelon plants grown in suppressive and nonsuppressive soil (Table 5-1). The bulk of the isolates, 110 F. oxysporum and 170 other microorganisms, were collected from suppressive soil, with nonsuppressive soil isolates collected primarily for comparative purposes. In addition to F. oxysporum, a total of 35 other fungal isolates included some other species of Fusarium, such as F. solani (Mart.)(Appel & Wr.) Snyd. & Hans. and F. equisetii (Corda) Sacc., in addition to species of Trichoderma, Penicillium, and Aspergillus. The 220 bacterial isolates consisted of 40 fluorescent pseudomonads, 30 actinomycetes, and 150 miscellaneous unidentified bacterial isolates.

Fifteen separate screening trials, each consisting of 20-30 individual isolate treatments, were required to screen all 395 isolates for their potential as antagonists to Fusarium wilt in CSMW soil. Disease levels in the pathogen-infested, microwave-treated control soil ranged from 55-100% wilt and averaged 81 ± 7% wilt over all the individual screening tests. Disease in the pathogen-infested, suppressive soil control ranged from 5-42% wilt, with an average of 28 ± 7% wilt over all tests. Disease levels in individual isolate treatments ranged from 5-100% wilt. To standardize the isolate effects and make

comparisons over several screening tests with varying disease levels, isolate effectiveness was expressed as the percent reduction of disease relative to infested CSMW control soil in each test. Isolates which were capable of substantially restoring suppressiveness and significantly ($P<0.05$) reducing disease in CSMW soil were considered effective antagonists in this soil. Isolates which reduced disease by 50% or greater and maintained low levels of wilt comparable to the suppressive soil were considered highly effective antagonists. Percent reduction of disease was a more reliable indicator of isolate effectiveness across screening tests than percent wilt or percent restoration of suppressiveness. All isolates effective in reducing disease in CSMW soil were subsequently tested in conducive field soil.

Effective isolates were found within all general organism types and in both suppressive and nonsuppressive soil (Table 5-1). However, isolates of F. oxysporum collected from suppressive soil were more effective than any other type of organism. Isolates of F. oxysporum from suppressive soil were responsible for the largest number and largest proportion of effective isolates (62%) than any other group, including isolates of F. oxysporum from nonsuppressive soil (30%). All other microorganism groups contained relatively few effective isolates; no major differences were observed in the proportion of effective isolates between suppressive and nonsuppressive soil (10-30%). In addition, the majority of isolates of F. oxysporum were highly effective in reducing disease, whereas organisms from other groups tended to be only marginally effective in reducing disease. The results from one typical screening trial are representative of the overall results of numerous tests; a high percentage of isolates of F. oxysporum and occasionally other organisms significantly reduced disease in microwave-treated soil (Figure 5-2; Summary of results from all tests are in Appendix A, Table A-10).

A total of 60 isolates of F. oxysporum and 62 isolates of other organisms that were effective antagonists in microwave-treated soil were screened in conducive field soil. Disease levels ranged from 55-96% wilt in the pathogen-infested control soil and averaged 73 ± 4% wilt over the eight separate screening trials conducted. Addition of individual antagonists resulted in disease levels ranging from 20-100% wilt, although relatively few of the isolates that were effective in microwave-treated soils showed any reduction of disease in field soil. Numerous isolates of F. oxysporum from suppressive soil significantly reduced disease, and constituted the vast majority of isolates effective in field soil. Wilt levels in treatments with effective antagonists ranged from 20-60% wilt. There was a wide variation among isolates of F. oxysporum from suppressive soil in their ability to reduce disease (Figure 5-3). Of a total of 51 isolates of F. oxysporum from suppressive soil tested, 18 significantly reduced disease in at least one field soil test. Of nine isolates from nonsuppressive soil, only one reduced disease, and this was only in one test. In general, bacteria, actinomycetes, and other fungal isolates were poor antagonists when tested individually in field soil. Out of 62 other organisms tested (42 from CSS soil, 20 from FGM soil), only 3 bacterial isolates from CSS soil showed a reduction of wilt in any test (Figure 5-3). All isolates that reduced disease in the first field soil test were screened in two or three additional field soil trials. The three bacterial isolates did not consistently reduce disease in these repeated tests. However, several isolates of F. oxysporum from CSS soil were found to consistently reduce Fusarium wilt in all field soil tests (Table 5-2). No isolates of fluorescent pseudomonads, actinomycetes, other bacteria, or other fungi resulted in significant disease reduction in multiple field soil tests. Thus, all isolates that were consistently effective as antagonists when added individually to field soils were isolates of F. oxysporum from the suppressive soil. Some individual isolates compared

favorably to suppressive soil regarding the reduction of disease relative to conducive field soil (28% wilt, 62% reduction) over all tests.

Characteristics of Successful Antagonists

Internal colonization of watermelon roots by the OM pathogen and other F. oxysporum, as determined by plating surface-disinfested roots, was compared for numerous potential antagonists. Due to the numbers of individual isolates involved, the colonization data were categorized according to organism type and whether the isolate was effective or not effective in reducing disease in microwave-treated soil (Table 5-3). In this way, root colonization characteristics for successful antagonists were compared as a group with unsuccessful antagonists to determine similarities, differences, and possible modes of action. Isolates of F. oxysporum, whether effective or not effective in reducing disease, in general, resulted in reduced root colonization by the OM pathogen compared to microwave-treated, control soil and all bacterial isolates tested. Colonization by the pathogen in both groups of antagonist F. oxysporum also was comparable to the colonization observed in the suppressive soil control. There was no difference among isolates of F. oxysporum which were effective or not effective in reducing disease regarding colonization by other F. oxysporum. For isolates of F. oxysporum added to microwave-treated soil, colonization by F. oxysporum other than the OM pathogen represented colonization by the antagonist, since all other F. oxysporum was eliminated by the microwave treatment. Thus, there was no difference in colonization by the potential antagonist for both effective and noneffective groups of F. oxysporum. Colonization by F. oxysporum also was higher in all treatments with F. oxysporum than in the suppressive soil controls. Effective bacterial isolates also resulted in reduced colonization by the

pathogen compared to noneffective isolates and the microwave-treated control soil groups. Wilt levels were comparable for effective antagonist groups and the suppressive soil controls, all of which were less than the noneffective isolates and microwave-treated control soil.

Comparisons of internal root colonization for similar antagonist groupings (although not all of the same isolates) in field soil tests gave similar results to those in microwave-treated soils (Table 5-4). Colonization by the OM pathogen was lower in treatments with isolates of F. oxysporum than in those with noneffective bacteria and infested field soil controls, but there was no difference between the effective and noneffective isolates of F. oxysporum. There was, however, a difference in colonization by other F. oxysporum between effective and noneffective isolates of F. oxysporum; higher colonization by F. oxysporum occurred with the noneffective group than with any other groups. Bacterial isolate results were similar to those observed in microwave soil, except for the very few (2) effective bacterial isolates found. Differences in wilt levels among the groups were similar to those observed in microwave-treated soil. Colonization data for individual isolates followed the same trends as for the overall groups; no individual, effective antagonistic isolate of F. oxysporum demonstrated different colonization characteristics than the others.

Vegetative compatibility analysis was conducted on about 30 isolates of F. oxysporum representing both effective and noneffective antagonists from suppressive and nonsuppressive soil. Only the four isolates that were already known to be pathogenic were compatible with previously established vegetative compatibility groups (VCGs) for F. oxysporum f. sp. niveum (Chapter 2). All four of these were weakly pathogenic on 'Crimson Sweet' watermelon and were in VCG 0081 (race 1). Two of these four isolates

did show some antagonistic potential in both microwave-treated and field soils, although they were not consistently effective in repeated field soil tests. No other isolates were compatible with pathogenic VCGs, nor were they compatible with each other; there were no common VCGs identified among any of the isolates, whether effective or not effective as antagonists.

Effective antagonists were isolated from bulk soil and in association with roots, as well as from soil that had been microwave-treated for 0, 30, and 60 s/kg soil (Table 5-5). In microwave-treated soil, the largest percent of effective isolates came from soil treated with a 30-s microwave exposure and the lowest from soil treated for 60 s/kg. While a high percentage of isolates from bulk soil were effective in microwave-treated soil, relatively few of these were effective in field soil (17%). Organisms from root area isolations, however, all had comparable proportions of effective isolates in field soils (39-43%), regardless of differences in the level of microwave treatment received or the percentages of effective isolates in microwave-treated soil. Because of the relatively few isolates of F. oxysporum that were collected from rhizosphere and rhizoplane dilution plates compared to those collected from surface-disinfested roots, accurate comparisons could not be made among the various root isolation methods. Similar percentages of effective antagonists appeared to be isolated with all three methods.

Induced Systemic Resistance

The split-root technique employed in this study was successful in separating the pathogen from potential antagonists in suppressive soil. Individual plants planted in the dual-pot system as controls demonstrated that only plants in the OM pathogen control side wilted. Individual plants as well as split-root plants showed no cross-contamination

on roots between the two pots; the OM pathogen was only isolated from roots on the side it was added to and abundant antagonists were only isolated from roots in the other pot. Treatment with an antagonistic isolate of F. oxysporum, which was highly effective in reducing disease in field soil tests, resulted in significantly less disease ($P<0.05$), based on the percentage of plants visually showing severe disease symptoms, than conducive soil treatments (Table 5-6). The suppressive soil treatment also had somewhat lower ($P<0.1$) disease levels than the conducive field soil treatment. However, when disease was assessed by the percentage of plants with the OM pathogen present in the vascular tissue of the above-ground stem sections, there were no differences among any of the treatments; nearly all plants had systemic infection regardless of treatment. A repeat of this experiment was of questionable validity due to low pathogen inoculum levels. The test produced very low levels of wilt (24-47% systemic infection) even in the pathogen controls and no differences among any of the treatments were detected.

Discussion

Nearly 400 microorganism isolates, representing a wide variety of indigenous soil microorganisms, were collected from the roots of watermelon plants grown in suppressive and nonsuppressive soils. Of these organisms, only specific isolates of F. oxysporum from the suppressive soil were consistently successful in reducing Fusarium wilt in a conducive field soil. Although numerous other organisms were capable of reducing disease in microwave-treated CSS soil and a few were effective in isolated field soil tests, none were more effective than F. oxysporum at any screening stage and none were consistently effective in repeated field soil tests.

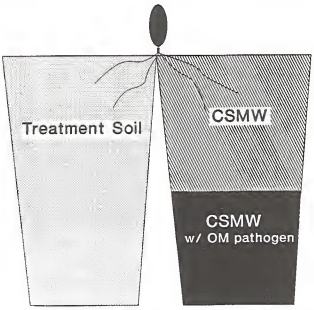


Figure 5-1. Diagram of watermelon plant in split-root pot assembly (Treatment soil=suppressive, monoculture soil; conducive, field soil; microwave-treated, suppressive soil; or microwave-treated, suppressive soil supplemented with an antagonistic isolate of Fusarium oxysporum not pathogenic on watermelon. CSMW=microwave-treated, suppressive soil; CSMW w/OM pathogen=microwave-treated soil with orange mutant pathogen added at 400 chlamydospores/g soil).

Table 5-1. Potential antagonistic organisms isolated from watermelon roots in suppressive and nonsuppressive soil and their ability to reduce disease in infested, microwave-treated, suppressive soil.

Organism group /Medium used[a]	Soil used[b]	Microorganism isolates: Total (#)	Reduced disease[c] (#)	Reduced disease[c] (%)	>50% Reduction of disease[d] (%)
F. oxysporum	CSS	110	68	62	55
/Komada	FGM	30	9	30	27
Other fungi	CSS	25	4	16	12
/PDA	FGM	10	1	10	0
Fluor. pseudomonads	CSS	30	7	23	10
/KMB	FGM	10	2	20	0
Other bacteria	CSS	120	29	24	12
/NA, TSA, KMB	FGM	30	7	23	3
Actinomycetes	CSS	20	5	25	8
/AWA	FGM	10	3	30	10

[a] Komada's (1975) selective medium was used for isolating F. oxysporum; all other fungi were isolated on potato dextrose agar (PDA) with tergitol and chlortetracycline added; flourescent pseudomonads and other bacteria were isolated on King's medium B (KMB) with penicillin, cyclohexamide, and novobiocin added (Sands and Rovira, 1970); all other bacteria were isolated on nutrient agar (NA) and 1/10 strength tryptic soy agar (TSA); actinomycetes were selected for on alkaline water agar (AWA), pH 10.5 (Ho and Ko, 1980).

[b] CSS=suppressive, 'Crimson Sweet' monoculture soil and FGM=nonsuppressive, 'Florida Giant' monoculture soil.

[c] Values represent the numbers and percentages of isolates within each group which resulted in a significant ($P<0.05$) reduction of disease (relative to pathogen-infested control soil) in microwave-treated soil screening tests. Chlamydospore inoculum of the orange mutant pathogen was added to treated soil at 200 cfu/g soil, followed by introduction of a conidial suspension of the antagonist, at 10^4 cfu/g soil for fungi and 10^5-10^7cfu/g soil for bacteria. Five 'Crimson Sweet' watermelon seeds were planted per pot (five pots/treatment) and disease was assessed as the percentage of wilted plants after 4 weeks of growth. Disease in pathogen -infested, control soil averaged 81 ± 7% wilt.

[d] Values represent the percentages of isolates in each group that reduced disease by more than 50% and maintained wilt levels comparable to suppressive soil controls.

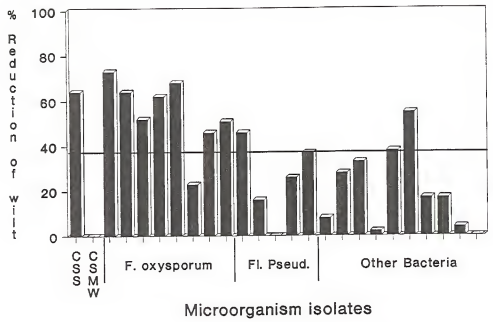
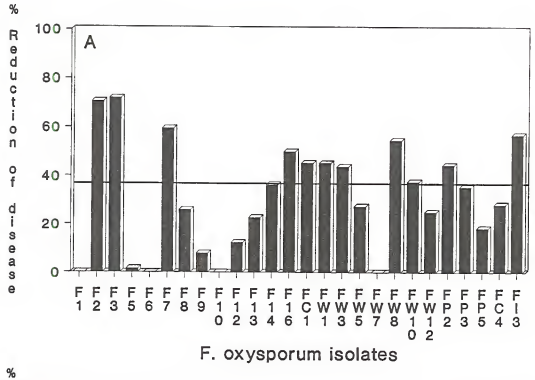


Figure 5-2. Percent reduction of Fusarium wilt by various potential antagonistic isolates of Fusarium oxysporum, fluorescent pseudomonads, and other bacteria in a typical screening trial in microwave-treated soil (CSS=pathogen-infested, suppressive soil control; CSMW=pathogen-infested, microwave-treated soil control). Horizontal line indicates the level of significant reduction of disease ($P<0.05$). All bars extending above this line represent isolates which significantly reduced disease. Average wilt in CSMW control soil was 80%.

Figure 5-3. Percent reduction of Fusarium wilt in conducive field soil screening tests by various bacteria and nonpathogenic isolates of Fusarium oxysporum collected from suppressive soil. A) Various isolates of F. oxysporum that were effective in reducing disease in microwave-treated soil; B) various bacterial isolates including fluorescent pseudomonads that were effective in reducing disease in microwave-treated soil. Horizontal line indicates the level of significant reduction of disease ($P<0.05$). All bars extending above line represent isolates which significantly reduced disease. Data for each isolate represents results from a single field soil test. Average wilt in untreated control soil was 72.5%.


% Reduction of disease
100
80
60
40
20
0
A
F 1
F 2
F 3
F 5
F 6
F 7
F 8
F 9
F 10
F 12
F 13
F 14
F 16
F C1
F W1
F W3
F W5
F W7
F W8
F W10
F W12
F P2
F P3
F P5
F C4
F I3
F. oxysporum isolates

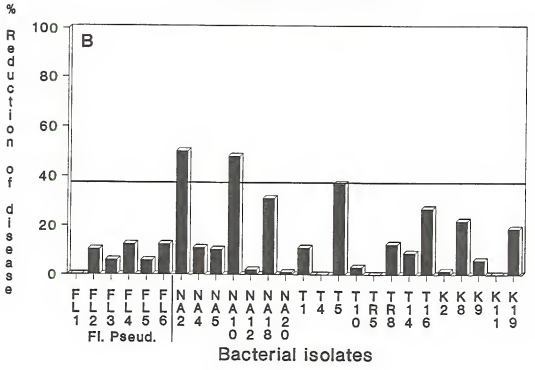

% Reduction of disease
100
80
60
40
20
0
B
FL 1
FL 2
FL 3
FL 4
FL 5
FL 6
NA 2
NA 4
NA 5
NA 10
NA 11
NA 28
NA 20
T 14
T 15
T 10
TR 5
T 18
T 14
T 16
K 12
K 8
K 9
K 11
K 19
Fl. Pseud.
Bacterial isolates

Table 5-2. Isolates of Fusarium oxysporum most effective in reducing disease in repeated antagonist screening tests.

Isolate	MW-treated soil tests[a]		Field soil tests[b]	
	% Wilt	% Reduction	% Wilt	% Reduction
CS30-RT2	25	73	23a[c]	65d
CS0-3l3	10	89	25a	60cd
CS0-RW1	40	37	31ab	60cd
CS30-RT7	31	68	34ab	51bcd
CS60-RP3	38	62	35ab	52bcd
CS30-RW5	45	51	38ab	42bc
CS0-RTN1	25	62	40ab	41bc
CS0-RT6	31	69	46b	38b
CSMW soil control	81	0	-	-
CSS soil control	-	-	28a	62d
LFC soil control	-	-	73c	0a

[a] Microwave-treated CSS soil was infested with chlamydospores of the OM pathogen at 200 cfu/g soil, followed by incorporation of a conidial suspension of the antagonist at 10^4 cfu/g soil. Percent reduction of disease was determined relative to the pathogen-infested control soil in each test.

[b] Field soil screening tests used a conducive field soil (LFC) infested with chlamydospores of the OM pathogen at 300 cfu/g soil, followed by introduction of a conidial suspension of the antagonist, at 10^4 cfu/g soil. Five 'Crimson Sweet' watermelon seeds were planted per pot (five pots/treatment) and disease was assessed as the percentage of wilted plants after 4 weeks of growth. Numbers represent means of two to three tests combined for each isolate. Values for controls are averages over all the screening tests conducted.

[c] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Table 5-3. Effect of antagonist treatments in microwave-treated suppressive soil on internal colonization of watermelon roots by Fusarium oxysporum f. sp. niveum and other F. oxysporum.

Treatment group[a]	# Isolates[b]	Colonies per 100 cm root[c]		% Wilt
		OM pathogen	F. oxysporum	
Effective F.oxysporum	25	4.33a[d]	10.38a	30a
Noneffective F.oxysporum	8	5.28a	10.80a	61b
Effective bacteria	10	10.28b	1.92c	34a
Noneffective bacteria	11	12.97c	0.80c	61b
MW-treated soil control	6	14.38c	0.37c	73c
Suppressive soil control	6	4.73a	6.86b	35a

[a] Data from a number of individual isolates were grouped together according to organism type and effectiveness in reducing disease ($P<0.05$) in microwave-treated soil.

[b] Values represent the numbers of isolates within each treatment group from which individual colonization data were compiled. The numbers for control treatments refer to the numbers of individual experiments from which data were compiled.

[c] Roots of 4-week-old plants were washed, surface-disinfested in 0.5% sodium hypochlorite for 1 min, rinsed, and embedded intact in Komada's (1975) medium. Four replications of four to six roots were used for each isolate. Root length was estimated by a line-intersect method (Tennant, 1975). Colonization of roots by the orange mutant (OM) pathogen strain of F. oxysporum f. sp. niveum and all F. oxysporum other than the OM pathogen was determined. Numbers represent the average colonization for all isolates in each group. Pathogen inoculum was added as chlamydospores at 200 cfu/g soil.

[d] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Table 5-4. Effect of antagonist treatments in conducive field soil on internal colonization of watermelon roots by Fusarium oxysporum f. sp. niveum and other F. oxysporum.

Treatment group[a]	# Isolates[b]	Colonies per 100 cm root[c]		% Wilt
		OM pathogen	F. oxysporum	
Effective F. oxysporum	14	9.15b[d]	5.39a	39b
Noneffective F. oxysporum	12	8.41b	8.33b	68c
Effective bacteria	2	8.91bc	3.28a	47b
Noneffective bacteria	6	12.79c	4.95a	75c
Field soil- w/pathogen	4	14.09c	4.94a	73c
Field soil- No pathogen	4	0.00a	4.41a	0a

[a] Data from a number of individual isolates were grouped together according to organism type and effectiveness in reducing disease ($P<0.05$) in microwave-treated soil.

[b] Values represent the numbers of isolates within each treatment group from which individual colonization data were compiled. The numbers for control treatments refer to the numbers of individual experiments from which data were compiled.

[c] Roots of 4-week-old plants were washed, surface-disinfested in 0.5% sodium hypochlorite for 1 min, rinsed, and embedded intact in Komada's (1975) medium. Four replications of four to six roots were used for each isolate. Root length was estimated by a line-intersect method (Tennant, 1975). Colonization of roots by the orange mutant (OM) pathogen strain of F. oxysporum f. sp. niveum and all F. oxysporum other than the OM pathogen was determined. Numbers represent the average colonization for all isolates in each group. Pathogen inoculum was added as chlamydospores at 300 cfu/ soil.

[d] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Table 5-5. Comparison of source and isolation conditions of isolates of Fusarium oxysporum effective in reducing disease in microwave-treated and field soils.

Isolation conditions[a]			Microorganism isolates			
			MW-treated soil test[b]		Field soil screening test[c]	
Soil	Sample type	Microwave treatment (s/kg soil)	# Effective /Total #	% Effective	# Effective /Total #	% Effective
CSS	Bulk soil	0	26/40	65	2/12	17
CSS	Root area	0	22/35	63	9/21	43
CSS	Root area	30	15/17	88	5/13	39
CSS	Root area	60	5/18	28	2/5	40

[a] All effective isolates were isolated from 'Crimson Sweet' suppressive (CSS), monoculture soil. Sample type refers to the sample material used, either bulk soil or from the root area, which includes isolations from the rhizosphere, rhizoplane, and surface-disinfested roots of watermelon plants. Microwave (MW) treatments of 0, 30, or 60 s/kg soil (2450 MHz, 700watts, at -0.01 MPa matric potential) were made prior to planting of watermelon.

[b] Isolate screening tests were conducted using microwave-treated CSS soil infested with the orange mutant pathogen at 200 cfu/g soil, followed by introduction of a conidial suspension of the antagonist at 10^4cfu/g soil. Five 'Crimson Sweet' watermelon seeds were planted per pot (five pots/treatment) and disease was assessed as the percentage of wilted plants after 4 weeks of growth. Isolates which reduced disease ($P<0.05$) relative to the pathogen-infested control were considered effective antagonists in this test.

[c] Conducive field soil (LFC) infested with chlamydospores of the orange mutant pathogen at 300 cfu/g soil was used to screen antagonists that had reduced disease in microwave-treated soil. Conidial suspensions of the antagonistic isolates were mixed into the soil at 10^4 cfu/g soil, and 'Crimson Sweet' watermelon were planted (five/pot, five pots/treatment). Plants were grown for 4 weeks and disease was assessed as the percentage of wilted plants.

Table 5-6. Development of Fusarium wilt in split-root watermelon plants with one half of each root system exposed to the pathogen and the other half exposed to conducive and suppressive soil treatments.

Treatment Soil[a]	Visual Symptoms[b] (% Wilt)	Systemic Infection[c] (% Plants)
MW-treated suppressive soil	81a[d]	92a
Conducive field soil	87a	100a
Suppressive soil	65ab	100a
F. oxysporum antagonist (CS30-RT7)	43b	87a

[a] Split-root tests consisted of four replications of five plants/treatment. The orange mutant pathogen was added to one side of a dual-pot setup at 400 cfu/g soil and the treatment soil filled the other pot. Antagonist treatment (CS30-RT7) consisted of a root-dip of one-half the root system in a conidial suspension of 10^6 cfu/ml prior to transplanting. Plants were grown for 5 weeks.

[b] Visual symptoms were assayed as the percentage of plants showing severe wilt symptoms.

[c] Systemic infection was represented by the percentage of plants in which the orange mutant pathogen was present within the vascular tissue. Determinations of systemic infection were made by plating surface-disinfested stem sections on Komada's (1975) medium.

[d] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

The wide discrepancy in effectiveness among individual nonpathogenic isolates of F. oxysporum indicates that specific antagonistic isolates are responsible for this suppression rather then a general population response attributable to all nonpathogenic strains of F. oxysporum. A larger proportion of effective antagonistic isolates of F. oxysporum were found in CSS than FGM soil, although even in suppressive soil effective antagonists accounted for a relatively small proportion of the total population of F. oxysporum. Isolates that are effective antagonists evidently possess specific traits, absent from the majority of isolates, that make them successful in reducing disease.

Although relatively few other species of Fusarium were tested, other studies have demonstrated F. oxysporum and, to a lesser degree, F. solani as the only important species involved in suppressiveness by Fusarium; other species, such as F. equisetii and F. roseum, showed little to no antagonistic activity (Alabouvette, 1986; Rouxel et al., 1979; Tamietti and Pramotton, 1990). The failure of any individual bacterial or fungal isolates other than F. oxysporum to effectively reduce disease in these tests is similar to the findings of Alabouvette and co-workers (Alabouvette, 1986; Alabouvette et al., 1985b; Louvet et al., 1981; Tamietti and Alabouvette, 1986) using similar screening tests with the suppressive soils of the Chateaurenard region of France.

Testing of the isolates in microwave-treated, suppressive soil was effective as a first step in the antagonist screening process. Using this biological assay, all organisms that were capable of reducing disease under the favorable conditions of this system were identified. This provided a substantial pool of potential antagonists, yet it succeeded in eliminating 40-90% of the original isolates from each organism category that would not be active in suppression. Alabouvette (1986, 1989) and others (Louvet, 1989; Rouxel et al., 1979) advocate this type of approach for determining the importance of various

potential antagonists in suppressive soils. Removal of suppression by microwave treatment, which leaves a large bacterial biomass (Chapter 3), provided a less disrupted environment than other soil treatments (autoclaving, fumigation), yet allowed a favorable environment for antagonists to operate. Only those organisms that have some antagonistic activity toward the pathogen or disease development are successful in this type of assay (Louvet, 1989). Thus, organisms that can re-establish suppressiveness to this soil are likely candidates for an active role in the suppressive soil. However, potential antagonists which were effective at reducing disease in microwave-treated soil were not always effective in field soil; the restoration of suppression to this microbiologically disrupted system did not necessarily translate into a corresponding role in disease suppression in the field. It is possible that reduction of disease in these two soils may, in some cases, involve different mechanisms. Competition provided by an organism capable of extensive colonization and virtual domination of the microflora in the reduced competition of a heat-treated soil may not be very important in a natural soil in which the antagonist may not multiply and may be but one small component of a complex microflora. This may be especially true for some of the bacterial isolates and primary colonizing fungi. Isolates of F. oxysporum, however, were the most effective antagonists in microwave-treated soil, as well as in field soil.

Other organisms which demonstrated some antagonistic activity in microwave-treated soil may play some role in disease suppression. These organisms may not be effective as antagonists individually, but they may enhance the overall suppressive effect of the primary antagonist. Such organisms may act in conjunction with F. oxysporum or other groups of antagonistic organisms and provide a background of general suppression in which the specific suppression by F. oxysporum operates (Alabouvette, 1989; Cook

and Baker, 1983; Louvet, 1989); such a contribution may only be apparent when all the proper components are present. Fluorescent pseudomonads have been shown to enhance the suppression provided by antagonism by F oxysporum in some soil systems (Alabouvette, 1989; Park et al., 1988). Various combinations of fluorescent pseudomonads, other bacteria, or other fungi which reduced disease in microwave-treated soil in this study may enhance suppression when added in conjunction with effective F. oxysporum antagonists in conducive soil. In preliminary experiments using a few combinations of different bacterial isolates added with and without an antagonistic isolate of F. oxysporum, no noticeable increase in disease suppression was observed (data not shown). However, these were very limited tests and the possibility of contributions to suppressiveness by other organisms needs to be investigated further. Although some individual isolates of F. oxysporum appeared to be just as effective as the untreated, suppressive soil in reducing disease, most isolates were not, indicating that other organisms may also contribute to this suppression. Differences were observed between populations of organisms in CSS and FGM soils in previous studies (Chapters 3 and 4) and some of these differences also may be involved in the overall suppressive response of this soil.

Inoculum concentrations of the potential antagonists in these screening tests were high, but not unreasonable; bacterial, actinomycete, and fungal isolates were added at levels which were comparable to their total populations in these soils (Chapter 3). However, isolates of F. oxysporum were added at levels higher than their natural populations (1-2 x 10^3 cfu/g soil in CSS soil). These levels were similar to those observed in other Fusarium wilt-suppressive soils (Alabouvette, 1986, 1989); part of the effectiveness of F. oxysporum as an antagonist in these soils was attributed to their large

populations. Similarly, in this study, competition between the pathogen and high populations of nonpathogenic isolates was studied for its effect on disease. Because the intent of this investigation was to identify all potential antagonists that could have a role in disease suppression, it was necessary to maintain antagonist population levels which would allow them to have a competitive advantage to ensure that antagonistic organisms were not overlooked due to insufficient population levels.

Any influence of differences between antagonist and pathogen populations of F. oxysporum in these tests is difficult to assess due to the different types of inoculum used for each (the pathogen was added as chlamydospores and the antagonist as conidia). Ideally, chlamydospore inoculum should be used for both pathogen and antagonist because it reflects the predominant residual propagule within the soil. Due to the vast numbers of F. oxysporum screened in these tests, however, it was not possible to prepare the more time-consuming chlamydospore inoculum for each antagonist. While chlamydospores are stable in the soil, conidia are ephemeral and may not translate directly into populations in the soil. Populations from conidial inoculum may decrease 10- to 50-fold within the first few weeks in soil; as an example, similar disease levels were produced with pathogen inoculum concentrations of 200 chlamydospores and 5000 conidia/g soil (Chapters 2 and 3). Thus, although the actual relationship of populations of the pathogen and antagonist are not known in these soils, the antagonist levels may not be much greater than the total population of F. oxysporum in the suppressive soil. Based on the variation in inoculum level produced throughout these tests with different isolates, antagonist effectiveness did not appear to be directly related to population levels; effective isolates were observed with substantially lower populations than noneffective isolates in soil dilution plate assays. Root colonization data also indicated

that competition from large populations of F. oxysporum was not consistently related to the reduction of disease. Although more thorough inoculum density studies will need to be conducted to determine the efficacy of the antagonistic isolates of F. oxysporum as biological control agents, the present study was sufficient to identify these organisms as important components active in the suppressive soil.

Effective antagonistic isolates of F. oxysporum were more likely to be found in the rhizosphere or colonizing the root than in the bulk soil, although isolates which could compete effectively in microwave-treated soil were just as prevalent in bulk soil as rhizosphere soil. The ability of F. oxysporum to effectively colonize soil has generally not been related to the ability to reduce disease in other studies (Tamietti and Pramotton, 1989; Louvet, 1989). This demonstrates the importance of the rhizosphere in antagonism to disease and may indicate a selection for antagonists in this area. Microwave treatments of 60 s/kg soil, which resulted in a marked decrease of suppression in CSS soil and reduced root colonization by F. oxysporum (Chapter 3), resulted in a lower percentage of isolates that were effective in reducing disease in microwave-treated soil. This could indicate that effective antagonists were more susceptible to heat treatment than were other isolates, although the percentage and level of effectiveness of the isolates that were effective in field soil was the same as for the other treatments.

Vegetative compatibility can be used as a means of subdividing natural populations of F. oxysporum and has been associated with such specific traits as virulence, colony size, isozyme patterns, and variation among nonpathogenic isolates (Correll et al., 1986b, 1987; Puhalla, 1985; Chapter 2). Vegetative compatibility was tested among a number of antagonistic isolates of F. oxysporum to determine if the traits responsible for effective antagonism were related to VCG in any way. Extreme diversity

has been observed among nonpathogenic isolates of F. oxysporum by others (Correll et al., 1986b; Elias and Schneider, 1987). Similarly, in the preliminary screening in this study, a large number of vegetative compatibility groups were present among the isolates tested, with no two isolates belonging to the same VCG. Thus, whatever trait or traits are related to the ability to reduce disease apparently were not associated with specific VCGs and are common to many VCGs. This is different than the association with VCG of traits for pathogenicity of F. oxysporum to watermelon, which are specifically associated with only three VCGs (Chapter 2). Due to this diversity, vegetative compatibility was not a useful tool for identifying or differentiating effective antagonists from other isolates of F. oxysporum.

The most prevalent theories to explain antagonism by nonpathogenic F. oxysporum are saprophytic competition at or near the root surface (Alabouvette, 1986; Lemanceau, 1989; Louvet 1989), parasitic competition for infection sites on the root (Schneider, 1984), and induced resistance to pathogen infection by prior colonization (Matta, 1989; Ogawa and Komada, 1986: Shimotsuma et al., 1972). As pointed out by Louvet (1989), these mechanisms are not necessarily exclusive of one another. Both saprophytic and parasitic competition may be occurring in the same soils. Induced resistance, although not yet implicated as a mechanism in suppressive soils (Louvet, 1989), may be active in environments where competition mechanisms also occur. Saprophytic competition for carbon and iron have already been shown to be important in the suppression of Fusarium wilt in Chateaurenard soils (Alabouvette, 1986; Lemanceau, 1989; Lemanceau et al., 1988) and soils of the Salinas Valley in California (Elad and Baker, 1985; Scher and Baker, 1982; Sneh et al., 1984). In the present

research, both competition at the root and induced resistance were studied for their possible roles in suppression by nonpathogenic F. oxysporum.

Root colonization characteristics of a number of isolates of F. oxysporum effective and not effective in reducing disease in the screening tests were compared to determine the role of competition at or on the root for the reduction of disease. In these tests, both effective and noneffective isolates of F. oxysporum were able to colonize roots at comparable levels and reduce colonization by the pathogen. Thus, reduction of disease was not related to the ability of an antagonist to extensively colonize roots or to reduce colonization by the pathogen. Although colonization of the root by the antagonist may be necessary for antagonism to occur, colonization alone did not necessarily result in lower disease. Schneider (1984) also observed that many isolates of F. oxysporum were capable of colonizing roots, but only some isolates were effective antagonists. For these isolates, reduction in root colonization by the pathogen was directly related to its effectiveness as an antagonist. This relationship led Schneider to conclude that disease suppression was due to parasitic competition for infection sites between antagonists and the pathogen. In the present study, reduction in root colonization by the pathogen caused by the antagonist was not sufficient to reduce wilt in either microwave-treated or field soil. The relative ability of an antagonist to compete with the pathogen for colonization of the roots (infection sites) was not related to its effectiveness as an antagonist. This is evidence against competition for infection sites on the root as the primary mechanism of suppression in this soil. Even isolates which did effectively compete with the pathogen for infection sites did not necessarily reduce wilt. These results, in addition to colonization data from previous studies (Chapters 3 and 4), also suggest that saprophytic competition is probably not the primary mechanism of disease suppression in these soils.

Several studies have shown that nonpathogenic or avirulent strains of F. oxysporum applied to roots can protect various hosts from Fusarium wilt when challenged by a virulent strain (Davis, 1967, 1968; Gessler and Kuc, 1982; Maraite, 1982; Matta, 1989; Ogawa and Komada, 1984, 1985, 1986; Wymore and Baker, 1982). This response has also been observed for Fusarium wilt of watermelon (Biles and Martyn, 1989; Martyn et al., 1990; Shimotsuma et al., 1972). Ogawa and Komada (1986) demonstrated that for sweet potato this protection was a result of an induced systemic resistance caused by previous infection by F. oxysporum. However, many other studies suggesting induced resistance do not adequately distinguish this response from competition at the roots, since both antagonist and pathogen treatments are normally applied to the same roots (Matta, 1989). For this reason, a split-root technique was used in this study to assay for induced systemic resistance as a mechanism of suppression by F. oxysporum in CSS soil. The split-root technique as used here was successful in physically separating the pathogen from antagonists, thus eliminating any interference with effects due to competition between pathogen and antagonist.

Although results of the split-root experiments did not clearly show an induced systemic resistance as the mechanism of suppression in CSS soil, there did appear to be some induced effect by colonization with an antagonistic isolate of F. oxysporum. There was evidence of a reduction or delay in symptom development, even though the antagonist did not prevent systemic infection by the pathogen. Treatments containing the suppressive soil or individual antagonist often showed a partial wilt, with the pathogen present only in the vascular bundles on one side of the plant. This may indicate limited movement of the pathogen in these plants. Maraite (1982), working with Fusarium wilt of muskmelons, also observed a delay in disease development caused by nonpathogenic

isolates of F. oxysporum in similar split-root experiments, although the effect was not as great as when the pathogen and antagonist were inoculated on the same root.

The split-root assay can only detect systemic responses, however, and can not identify induced resistance if it is only local in nature. Several reports on induced resistance to Fusarium wilt diseases imply a systemic response (Biles and Martyn, 1989; Davis, 1967; Hillocks, 1986; Maraite, 1982; Ogawa and Komada, 1986), while others have suggested that the induced effects are localized in the surroundings of tissues inoculated with the antagonist (Matta, 1989; Shimotsuma et al., 1972). Local induced resistance may require numerous infections by the antagonist throughout the root system to effectively protect the root from attack by the pathogen. Such responses cannot be separated from competition effects in most assays and would not be detected in a split-root assay. It is possible that even a systemic response may not effectively translocate from the treated half of the roots over to the untreated roots as required in split-root experiments. Thus, although split-root tests can be helpful in determining the presence of a systemic response, they can not detect all types of induced resistance

The time between inoculation with the antagonist and challenge by the pathogen may be critical for identifying induced systemic resistance. In these experiments, this time period could not be accurately determined, although it is estimated that the root would grow 2-3 days before being infected by the pathogen. Most other studies have determined that the time necessary to allow induction processes to occur is somewhere between 24 and 72 hours (Biles and Martyn, 1989; Davis, 1967; Hillocks, 1986; Matta, 1989; Shimotsuma et al., 1972). A few studies have indicated even longer induction periods may be necessary (Dean and Kuc, 1986; Gessler and Kuc, 1982).

Proposed mechanisms responsible for induced resistance to Fusarium wilt include activation of vascular occlusion, antifungal compounds, growth regulators, and stress- or injury-related responses (Beckman, 1987; Matta, 1989). Hillocks (1986), working with an induced resistance to Fusarium wilt of cotton, concluded the mechanism to be vascular occlusion induced by a nonpathogenic strain of F. oxysporum, which limited the movement of the pathogen within the vascular tissue. Symptom reduction was associated with reduced populations of the pathogen in the stele tissue of the stem. Beckman and co-workers (Beckman and Halmos, 1962, Beckman et al., 1982) observed that some nonpathogenic strains of F. oxysporum induced more extensive vascular occlusion in banana and tomato than pathogens did. Thus, in these studies the pathogen was found in the vascular tissue of most plants, but the population and location of the pathogen within the stele was limited, resulting in a delay or reduction in symptoms. Others also have reported that protection by induced resistance results primarily in a reduction or delay in symptoms (Biles and Martyn, 1989; Davis, 1967, 1968; Maraite, 1982) and can be overcome by increases in pathogen inoculum (Martyn et al., 1990). Thus, the mechanism of induced resistance probably involves reducing or restricting the extent of pathogen colonization or movement within the vascular tissue rather than in the prevention of infection or initial colonization of the roots. This is consistent with the colonization data observed in the present study, wherein colonization of roots by the pathogen was the same for effective and noneffective isolates of F. oxysporum, but effective antagonists resulted in a reduction in symptom development. Since this reduction was observed to some degree even in the split-root tests, induced resistance may have contributed to limiting the movement and possibly reducing the population of the pathogen in the vascular tissue. Although the results from the split-root tests in this

study are only preliminary, the possibility of an induced resistance does exist and further investigation of induced resistance as a mechanism in this and other suppressive soils is warranted.

The results of this study, along with the previous work (Chapters 3 and 4), support the conclusion that indigenous isolates of F. oxysporum not pathogenic on watermelon appear to be the dominant antagonists and primary organisms responsible for the suppression of Fusarium wilt of watermelon in the 'Crimson Sweet' suppressive monoculture soil, although other organisms may contribute to this suppression. Individual isolates of F. oxysporum were more effective than others in reducing disease, but no specific traits related to effectiveness could be identified. There was no evidence for competition as the active mechanism of suppression by F. oxysporum, but preliminary tests indicate that some type of induced resistance may be occurring and requires further investigation. At this point, it is difficult to assess the effective antagonistic isolates of F. oxysporum identified in this study regarding their potential as biological control agents. Specific isolates of F. oxysporum have been used to control Fusarium wilt of tomato in field tests (Alabouvette, 1989) with mixed results. Generally, addition of mass quantities of an individual antagonist is not practical or effective in the field. However, combining moderate additions of effective antagonists with the cultivation of varieties which promote the development of these antagonists in the rhizosphere (such as 'Crimson Sweet'), may provide a more stable and effective means of biological control.

CHAPTER 6
SUMMARY AND CONCLUSIONS

This research has attempted to provide a basic understanding of the characteristics, organisms, and mechanisms responsible for soil suppressiveness of Fusarium wilt of watermelon in an unique disease-suppressive soil. The suppressive soil was developed through monoculture of watermelon cultivar 'Crimson Sweet' in the field. This cultivar-specific induction of suppression and its development in a soil type which is normally highly conducive to wilt distinguishes this suppressiveness from most other Fusarium wilt-suppressive soils reported.

Soils which are suppressive to disease are important to study because they may represent an effective, naturally occurring biological control system. Through careful analysis of the conditions, processes, and interactions responsible for disease suppression in these soils, much insight may be gained on how and why they are suppressive. Information provided by such analyses can then be used to incorporate these biological control principles into a disease control system within a variety of agricultural settings. In this study, the approach to accomplish this general objective focused primarily on comparing the ecology of the pathogen in the suppressive soil to that in other similar soils which were conducive to the disease. In this way characteristics and interactions unique to the suppressive soil could be identified and analyzed for their relationship to suppressiveness. Ultimately, some of the organisms and mechanisms

responsible for suppression, as well as an understanding of how and why they function, would be determined.

Vegetative compatibility was investigated as a means to identify and differentiate isolates of Fusarium oxysporum f. sp. niveum. Isolates from Florida and many locations from around the world were tested for pathogenicity and used to determine vegetative compatibility groups (VCGs) on the basis of heterokaryon formation by nitrate-nonutilizing mutants. All isolates of F. oxysporum f. sp. niveum tested belonged to one of three distinct VCGs; these isolates were incompatible with isolates not pathogenic on watermelon. Race 1 isolates were contained in two VCGs (0080 and 0081), while all race 2 isolates comprised a third VCG (0082). The occurrence of race 2 in Florida was also verified in this study. This study demonstrated that vegetative compatibility can be used to efficiently distinguish F. oxysporum f. sp. niveum from other isolates of F. oxysporum. This technique enabled quicker, easier, and more consistent identification of pathogenic races than was previously possible and also provided clarification of the distinctions between these races.

An additional use for this technique was to be to determine the population and race identification of the pathogen within the suppressive and conducive field soils used throughout this research. This would have provided valuable information on the differences in pathogen populations among the field soils. However, due to the small proportion of pathogenic isolates relative to other F. oxysporum in these soils, the number of isolates required to make an accurate assessment of any differences among pathogen populations in these soils was prohibitive. For the vegetative compatibility study, 60 isolates of F. oxysporum were collected from the suppressive, 'Crimson Sweet' monoculture (CSS) soil as well as from the nonsuppressive, 'Florida Giant' monoculture

(FGM) soil. Of these, only three isolates from CSS soil and five from FGM soil were pathogens. Therefore, to make accurate comparisons of pathogen populations between these soils, hundreds of isolates of F. oxysporum would be needed. Although VCG analysis is somewhat quicker and easier than pathogenicity tests, it still requires culturing and producing nitrate-nonutilizing mutants for every isolate. This technique is still too labor-intensive to enable it to function as a quick assay of pathogens within field soils. Thus, it has not yet been determined what role, if any, the distribution and racial composition of the pathogen has in disease-suppressiveness within these soils. Recently, an improved technique for an assay of this type has been proposed by Hopkins and Lobinske (1990). A highly susceptible watermelon cultivar is used in this technique to selectively recover pathogenic isolates from the soil, and races are determined subsequently by vegetative compatibility tests.

To gain a better understanding of the ecology of the pathogen in the suppressive soil, population dynamics and chlamydospore germination were monitored in four soils suppressive and conducive to Fusarium wilt. Colonization of watermelon roots by the pathogen, other indigenous Fusarium oxysporum, and various other microorganism groups also was monitored. In addition, the effect of successive plantings of watermelon cultivars on the populations of the pathogen, indigenous F. oxysporum, and other microorganisms was determined. The effect of successive planting on watermelon root colonization by the pathogen and indigenous F. oxysporum also was evaluated. In all of these tests, an orange-colored mutant isolate of the pathogen, which was comparable to the wild-type pathogen regarding growth in culture, pathogenicity, and root colonization, was used to differentiate the pathogen from indigenous F. oxysporum.

Population dynamics of the pathogen demonstrated some differences among the various soils. Pathogen populations remained stable in CSS soil over a 6-month period, with no substantial increase or decrease over time, whereas pathogen populations did increase somewhat when initially added to conducive soils. Suppressive soil maintained lower pathogen populations than conducive soils, even when planted to susceptible watermelon cultivars. However, pathogen populations in the suppressive soil were comparable to those in a nonsuppressive, monoculture soil at all stages of this research.

Suppressiveness was not associated with inhibition of pathogen chlamydospore germination or increased fungistasis in CSS soil, which is in contrast to what was observed with most other Fusarium wilt-suppressive soils studied (Alabouvette, 1986; Alabouvette et al., 1985b; Louvet et al., 1981; Cook and Baker, 1982; Smith, 1977). This suggests that a general suppression of the pathogen due to nutrient competition from a large diverse microbial biomass, which is evident in many suppressive soils, is not an important factor in CSS soil. Thus, the 'Crimson Sweet' suppressive soil was shown to be quite different from many other suppressive soils, not only in its development and characteristics, but also apparently in the mechanism involved.

Measurements of surface and internal colonization of watermelon roots by the pathogen and indigenous F. oxysporum were not consistently related to suppressiveness. There were no significant differences in root colonization by F. oxysporum between CSS and FGM soils in any of the tests, although there were differences when compared to conducive soils. There also was no relationship between the ratio of colonization by the pathogen to colonization by indigenous strains of F. oxysporum and suppressiveness. Even after successive plantings of watermelon cultivars, root colonization levels were not consistently related to disease suppression. These results indicated that large total

populations of indigenous F. oxysporum competing with the pathogen in the rhizosphere, as reported by Alabouvette (1986), were not directly responsible for suppression in these soils. This suggested that specific antagonistic strains rather than general populations of F. oxysporum may be involved in suppression.

Populations of various groups of microorganisms were affected by planting to different watermelon cultivars. Estimates of general populations of bacteria and actinomycetes in the soil and on watermelon roots were generally slightly higher in CSS soil than in the other soils; the largest difference occurred in fluorescent pseudomonad populations. Successive plantings of watermelon cultivars resulted in increases in the general prokaryotic microorganism populations in all soils. Planting to cultivar 'Crimson Sweet' resulted in significant increases in total populations of bacteria, actinomycetes, and fluorescent pseudomonads compared to planting with cultivar 'Florida Giant' in some soils. Successive plantings of watermelon also had significant effects on populations of F. oxysporum. When planted to the susceptible cultivar 'Florida Giant,' pathogen populations tended to increase with successive planting, while populations of indigenous F. oxysporum did not change significantly. When planted to 'Crimson Sweet,' pathogen populations did not change significantly, but populations of indigenous F. oxysporum tended to increase with successive plantings. Thus, cultivar 'Crimson Sweet' tended to promote the development of nonpathogens over the pathogen, whereas 'Florida Giant' tended to promote pathogen development over nonpathogens. Total fungal populations, other than F. oxysporum, were not affected by planting to watermelon. These results suggest that cultivar differences can change the composition of the rhizosphere microflora either by enhancing general population levels or selectively favoring the growth of certain groups of organisms, and that these changes may be related to disease suppression.

Using the information gained from these ecological studies on population dynamics and root colonization in the suppressive and conducive soils under various conditions, the final stage of the study involved identifying the specific antagonistic organisms active in the suppressive soil. Potential antagonists were isolated from the roots of watermelon growing in suppressive soil and evaluated for their possible roles in suppressiveness. Since their populations tended to increase with successive plantings of cultivar 'Crimson Sweet,' special emphasis was placed on indigenous strains of F. oxysporum and fluorescent pseudomonads. Nearly 400 isolates of microorganisms, including bacteria, actinomycetes, and fungi, were screened for their ability to restore suppression to microwave-treated, CSS soil and to induce suppressiveness in a conducive field soil. Specific isolates of F. oxysporum from suppressive soil were the only antagonists consistently effective in reducing disease (35-75% reduction) in both microwave-treated and field soils, although a few other organisms were effective in isolated tests. Thus, it appears that indigenous isolates of F. oxysporum not pathogenic on watermelon are the primary organisms responsible for suppressiveness in CSS soil, although other organisms may enhance this suppressive effect. Not all isolates of F. oxysporum were effective, however; only 18 of 51 isolates from suppressive soil and one of nine isolates from nonsuppressive soil reduced disease in field soil.

Isolates effective in reducing disease tended to colonize roots extensively and reduce root colonization by the pathogen. However, many isolates of F. oxysporum which were not effective in reducing disease also had the same pattern of root colonization. Thus, reduction of disease was not related to the ability of an isolate to extensively colonize the root or reduce colonization by the pathogen. This does not support saprophytic competition at or near the root surface (Alabouvette, 1986) or parasitic

competition for infection sites on the root (Schneider, 1984) as the primary mechanism for suppression by F. oxysporum in CSS soil. It was not possible to identify specific characteristics of effective antagonists.

Split-root experiments were conducted to investigate the possibility of an induced systemic resistance caused by infection by nonpathogenic isolates of F. oxysporum as a mechanism of suppression. A reduction or delay in symptom development in one test in suppressive soil and with an antagonistic isolate of F. oxysporum indicated that induced resistance is a possible mechanism for suppressiveness in this soil. Although these tests were only preliminary, an induced resistance response is consistent with the population and colonization data throughout this research.

This research, taken as a whole, has evaluated many of the ecological characteristics related to the pathogen in this unique suppressive soil, has identified the primary organism responsible for suppression, and has investigated the possible mechanisms of suppression. Areas which require further investigation to better understand how and why this soil suppresses disease include determination of the active mechanism of suppression, the specific traits which make an antagonist effective, and the mechanism by which 'Crimson Sweet' promotes suppressiveness, among others. Although there are still many questions that need to be answered, this research has contributed a solid base of information on the 'Crimson Sweet' suppressive, monoculture soil and its relationship to other suppressive soils. Through analysis of the ecological interactions of the pathogen and other microorganisms and the evaluation of potential antagonistic organisms, this research has provided a better understanding of the nature and functioning of this suppressive soil system. The results suggest that monoculture of watermelon cultivar 'Crimson Sweet' changes the ecological balance of the soil by

selectively enhancing antagonistic isolates of F. oxysporum not pathogenic to watermelon, as well as other microorganisms. Information generated on the characteristics, relationships, organisms, and mechanisms active in this soil can now be used to provide the groundwork toward the development of a practical system of biological control of Fusarium wilt of watermelon and possibly other Fusarium wilt diseases.

APPENDIX A
ADDITIONAL DATA FROM REPEATED EXPERIMENTS

Table A-1. Wilt development in different watermelon cultivars planted in soil infested with various isolates of Fusarium oxysporum f. sp. niveum (Results of imported isolate test 1, which were combined with test 2 in Table 2-2).

Isolate	VCG	Race	Percent wilt on indicated cultivar[a]						
			FG[b]	ChG	CS	SL	DL	CalG	Mean
18467	0080	1	49a[c]	14a	60ab	8a	14a	8a	25
O-1132	0080	1	63ab	35bc	76ab	20ab	32b	23ab	41
O-1128	0080	1	73abcd	45bc	70ab	25abc	24ab	23ab	43
O-936	0080	1	85cde	43bc	58ab	36bc	32b	17ab	45
O-1182	0080	1	67abc	28ab	67ab	48cd	47bc	21abc	46
O-1130	0080	1	87cde	43bc	68ab	47bcd	42b	23abc	52
FG85-1	0080	1	95ef	53cd	78ab	42bcd	25ab	18ab	53
O-987	0080	1	74bcd	58cd	83bc	42bcd	44b	25abc	54
O-974	0080	1	97ef	74d	90c	48cd	52bc	33bc	66
O-1210	0080	1	100ef	88e	88c	48cd	46bc	51d	70
FG85-2	0081	1	90de	36bc	54a	43bcd	50bc	55d	55
FG85-15	0081	1	93ef	46bc	57ab	64d	35b	48cd	57
TX-X1D	0082	2	86def	60cd	72ab	88e	75d	84e	78
CS85-4	0082	2	93ef	100f	100d	91e	75d	86e	91
Cultivar averages			82	51	73	46	42	37	

[a] Percent wilt indicates the incidence of Fusarium wilt after 4 weeks.

[b] Cultivars used were 'Florida Giant'(FG), susceptible to Fusarium wilt; 'Charleston Gray'(ChG) and 'Crimson Sweet'(CS), moderately resistant; 'Sugarlee'(SL), 'Dixielee'(DL), and 'Calhoun Gray'(CalG), highly resistant.

[c] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Means were based on four replicate pots of 10 plants each for each isolate/cultivar combination. Analysis was conducted on actual incidence data. Inoculum consisted of conidia added to the soil at 5×10^3/g soil.

Table A-2. Wilt development in different watermelon cultivars planted in soil infested with various isolates of Fusarium oxysporum f. sp. niveum (Results of imported isolate test 2, which were combined with test 1 in Table 2-2).

Isolate	VCG	Race	Percent wilt on indicated cultivar[a]						Mean
			FG[b]	ChG	CS	SL	DL	CalG	
O-1132	0080	1	60a[c]	16a	8a	0a	0a	0a	14
18467	0080	1	71ab	29ab	13ab	10ab	2a	5a	22
O-1128	0080	1	74ab	19ab	19ab	8ab	13ab	8a	24
O-1210	0080	1	88bc	25ab	25ab	7ab	13ab	8a	29
O-987	0080	1	91bc	58cde	18ab	9ab	5ab	11a	32
O-936	0080	1	96c	73de	28ab	9ab	8ab	9a	32
FG85-1	0080	1	95c	48bcd	22ab	39cd	8ab	10a	37
O-1182	0080	1	88bc	30ab	15ab	47de	26bc	15ab	37
O-1130	0080	1	82bc	64de	37abc	20abc	18ab	10a	39
O-974	0080	1	100c	57cde	29abc	27abcd	48d	21ab	47
FG85-2	0081	1	100c	40abcd	30abc	71f	44cd	43c	55
FG85-15	0081	1	96c	56cde	20ab	75f	56de	35bc	56
TX-X1D	0082	2	83bc	76e	53cd	65ef	64de	63d	67
TX-HC3	0082	2	96c	78e	74d	86f	70e	67d	80
CS85-4	0082	2	100c	80e	74d	76f	59de	64d	75
Cultivar averages			88	53	30	33	35	31	

[a] Percent wilt indicates the incidence of Fusarium wilt after 4 weeks.

[b] Cultivars used were 'Florida Giant'(FG), susceptible to Fusarium wilt; 'Charleston Gray'(ChG) and 'Crimson Sweet'(CS), moderately resistant; 'Sugarlee'(SL), 'Dixielee'(DL), and 'Calhoun Gray'(CalG), highly resistant.

[c] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Means were based on four replicate pots of 10 plants each for each isolate/cultivar combination. Analysis was conducted on actual incidence data. Inoculum consisted of conidia added to the soil at 5×10^3/g soil.

Table A-3. Wilt development in different watermelon cultivars planted in soil infested with isolates of Fusarium oxysporum f. sp. niveum from Florida (Florida isolate test).

Isolate	VCG	Race	Percent wilt on indicated cultivar[a]						
			FG[b]	ChG	CS	SL	DL	CalG	Mean
FG85-1	0080	1	97ab[c]	87cd	71ab	77ab	63ab	43ab	73
CS85-1	0080	1	94ab	85cd	82bc	72ab	44a	31a	68
FG85-20	0081	1	100b	38a	65ab	67a	68bc	40ab	63
FG85-2	0081	1	88a	68bc	57a	70a	58ab	50b	65
FG85-15	0081	1	97ab	59ab	67ab	76ab	70bcd	53b	70
JMC-71	0081	1	87a	72bc	69ab	76ab	62bc	51b	70
CS85-4	0082	2	97ab	92cd	86bc	84ab	78cd	90c	87
CS85-15	0082	2	100b	100d	95c	91b	86d	82c	92
Cultivar Averages			95	74	75	77	66	55	

[a] Percent wilt indicates the incidence of Fusarium wilt after 4 weeks.

[b] Cultivars used were 'Florida Giant'(FG), susceptible to Fusarium wilt; 'Charleston Gray'(ChG) and 'Crimson Sweet'(CS), moderately resistant; 'Sugarlee'(SL), 'Dixielee'(DL), and 'Calhoun Gray'(CalG), highly resistant.

[c] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Means were based on four replicate pots of 10 plants each for each isolate/cultivar combination. Analysis was conducted on actual incidence data. Inoculum consisted of conidia added to the soil at 5×10^3/g soil.

Table A-4. Average percent wilt of various watermelon cultivars caused by isolates of Fusarium oxysporum f. sp. niveum in three vegetative compatibility groups.

VCG	Race	Percent wilt on indicated cultivar[a]						
		FG[b]	ChG	CS	SL	DL	CalG	Mean
Imported isolate test 1[c]								
VCG 0080	1	79a[d]	49a	75b	39a	36a	24a	50
VCG 0081	1	91a	41a	55a	53a	42a	51b	56
VCG 0082	2	89a	80b	86c	89b	75b	85c	84
Imported isolate test 2[e]								
VCG 0080	1	84a	43a	21a	18a	14a	10a	32
VCG 0081	1	98a	48a	25a	73b	50b	39b	57
VCG 0082	2	93a	78b	64b	76b	65c	62c	73

[a] Percent wilt indicates the incidence of Fusarium wilt after 4 weeks.

[b] Cultivars used were 'Florida Giant'(FG), susceptible to Fusarium wilt; 'Charleston Gray'(ChG) and 'Crimson Sweet'(CS), moderately resistant; 'Sugarlee'(SL), 'Dixielee'(DL), and 'Calhoun Gray'(CalG), highly resistant.

[c] Wilt averages for individual isolates were grouped by VCG. Isolates used were ATCC 18467, O-936, O-974, O-978, O-1128, O-1130, O-1132, O-1182, O-1210, and FG85-1 (VCG 0080); FG85-2 and FG85-15 (VCG 0081); and TX-X1D, TX-HC3, and CS85-4 (VCG 0082).

[d] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Means were based on four replicate pots of 10 plants each for each isolate/cultivar combination. Analysis was conducted on actual incidence data. Inoculum consisted of conidia added to the soil at 5×10^3/g soil.

[e] The same isolates, inoculum level, and procedures as in the previous test were used.

Table A-5. Comparison of orange mutant isolates with wild-type parent isolate (FG85-1) of Fusarium oxysporum f. sp. niveum for growth characteristics, root colonization, and pathogenicity (Initial tests on all orange mutant isolates).

Isolate	Radial growth[a] (mm)	Conidium production[b] (cfux10^6/ml)	Mycelium mass[c] (mg)	Root colonization[d] (cfux10^4/g)	Pathogenicity[e] (% wilt) Conidial inoculum	Pathogenicity[e] (% wilt) Chlamydospore inoculum
FG85-1	38.9a[f]	1.50ab	96a	27.1a	60a	92a
FG-OR1	36.2a	2.54c	100a	3.0a	27cd	81a
FG-OR2	29.9b	1.07a	84a	2.9a	23d	38d
FG-OR3	38.0a	3.52d	95a	20.6a	61a	92a
FG-OR4	14.4d	-	-	-	-	-
FG-OR5	26.1c	1.20ab	81a	3.2a	20d	44b
FG-OR6	36.9a	1.75bc	95a	86.8b	70a	81a
FG-OR7	30.4b	0.75a	89a	6.2a	42bc	56b
FG-OR8	37.9a	2.13c	84a	38.8ab	50ab	83a

[a] Radial growth was measured on PDA plates after 7 days at 26°C (Average of three experiments, each with four replicate plates/isolate).

[b] Conidium production was estimated from 10 ml conidium suspensions made from 7-day-old PDA cultures (Average of two experiments with four replications/isolate).

[c] Isolates were grown in liquid medium (Netzer, 1976) for 5 days at 26°C. Means represent the dry weight of mycelial mats harvested on filter paper (Average of two experiments with four replications/isolate).

[d] Weighed root samples (four/isolate) of cultivar 'Florida Giant' were shaken in sterile water 20 minutes and the rootwash suspension dilution-plated on Komada's (1975) medium. Conidial inoculum of 5 x 10^3 cfu/g soil was used in all tests.

[e] Pathogenicity was measured as the incidence of Fusarium wilt on cultivar 'Florida Giant' in a microwave (MW)-treated soil (2 min/kg soil at -0.01 MPa). Conidial inoculum of 5 x 10^3 cfu/g soil and chlamydospore inoculum of 200 cfu/g soil were used (four replicate pots of five plants/test).

[f] Means within columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Table A-6. Microorganism populations on the roots of watermelon cultivars 'Crimson Sweet' and 'Florida Giant' in four different soils (Combined data for cultivars used in Figure 3-4).

Soil type[a]	Cultivar[b]	Log cfu / g root[c]				
		Bacteria	Actinomycetes	Fluorescent pseudomonads	Other pseudomonads	Fungi
CSS	CS	8.74c[d]	8.00b	5.81b	6.40c	4.50b
FGM	CS	8.36b	7.87b	5.40b	6.30bc	4.14a
LFC	CS	7.95a	6.96a	4.04a	5.91a	4.62b
CSMW	CS	7.97a	7.29a	3.24a	5.95ab	4.14a
CSS	FG	8.69b	8.11b	6.20c	6.10a	3.94a
FGM	FG	8.72b	7.99b	5.30b	6.25a	4.06a
LFC	FG	8.64b	7.25a	3.26a	6.04a	4.93b
CSMW	FG	8.24a	7.30a	3.29ab	6.06a	3.58a

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive); LFC=Leesburg fallow conducive soil; CSMW=microwave-treated, CSS soil (conducive).

[b] Watermelon cultivars used were 'Crimson Sweet' (CS), moderately resistant to Fusarium wilt of watermelon and inducer of soil suppressiveness, and suceptible cultivar 'Florida Giant' (FG).

[c] Estimates of microorganism populations were made by sonication of roots from 3-week-old plants in sterile water for 5 min and the resulting suspensions were dilution-plated on various agar media. Four replications of two roots each were used. Bacterial populations were estimated on nutrient agar and 1/10 strength tryptic soy agar; actinomycete populations were estimated on alkaline water agar; and pseudomonad populations were estimated on King's medium B with penicillin, cyclohexamide, and novobiocin added. Plates were examined under UV light and fluorescent pseudomonads identified by the production of diffusible fluorescent pigment. All other colonies resembling pseudomonads on these plates were counted as other pseudomonads. Fungal populations were estimated on potato dextrose agar with tergitol and chlortetracycline added.

[d] Means within columns for each cultivar followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test. Analysis between cultivars revealed no significant differences or interactions.

Table A-7. Microorganism populations on 'Crimson Sweet' watermelon roots in two soils exposed to varying microwave treatments (Repeat of experiment in Table 3-4).

Soil type[a]	Microwave exposure[b] (s/kg soil)	Log cfu / g root[c]				
		Bacteria	Actinomycetes	Fluorescent pseudomonads	Other pseudomonads	Fungi
CSS	0	8.50a[d]	7.34ab	5.86ab	6.68d	4.51b
FGM	0	8.34bc	7.59abcd	5.69a	6.04bc	4.62b
CSS	30	8.60d	7.40abc	6.13b	5.63b	4.63b
FGM	30	8.29ab	7.69d	6.06b	6.21c	4.44b
CSS	60	8.46bc	7.48abcd	5.98ab	5.79bc	4.44b
FGM	60	8.49bc	7.47a	5.91ab	6.05c	4.52b
CSS	90	8.50d	7.31a	6.62c	5.01a	3.93a
FGM	90	8.21a	7.65cd	6.47c	5.90bc	4.51b

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive).

[b] Duration of microwave exposure (s/kg soil) (2450 MHz, 700 watts) at -0.01 MPa matric potential. Following microwave treatment all soils were infested with an orange mutant pathogen at 200 cfu/g soil.

[c] Estimates of microorganism populations were made by sonication of roots from 3-week-old plants in sterile water for 5 min and the resulting suspensions dilution-plated on various agar media. Four replications of two roots each were used. Bacterial populations were estimated on nutrient agar and 1/10 strength tryptic soy agar; actinomycete populations were estimated on alkaline water agar; and pseudomonad populations were estimated on King's medium B with penicillin, cyclohexamide, and novobiocin added. Plates were examined under UV light and fluorescent pseudomonads identified by the production of diffusible fluorescent pigment, and other colonies resembling pseudomonads on these plates were also counted. Fungal populations were estimated on potato dextrose agar with tergitol and chlortetracycline.

[d] Means in columns followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Table A-8. Microorganism populations on 'Crimson Sweet' watermelon roots in two soils exposed to varying microwave treatments and overall population averages (Combined results of Table A-7 and Table 3-4).

Soil type[a]	Microwave exposure[b] (s/kg soil)	Log cfu / g root[c]				
		Bacteria	Actinomycetes	Fluorescent pseudomonads	Other pseudomonads	Fungi
CSS	0	8.27ab[d]	7.30a	5.68ab	6.18ab	4.46ab
FGM	0	8.03a	7.49a	5.43a	6.14ab	4.58ab
CSS	30	8.54b	7.49a	5.83b	5.85ab	4.44ab
FGM	30	8.16a	7.43a	5.53a	6.27b	4.75b
CSS	60	8.17a	7.39a	5.59ab	5.75a	4.22a
FGM	60	8.14a	7.29a	5.21a	6.14ab	4.77b
CSS	90	8.34b	7.32a	6.43c	5.83a	3.79a
FGM	90	8.04a	7.38a	5.82a	6.27b	4.63b
2-way ANOVA and overall averages.						
CSS	-	8.33b	7.37a	5.88b	5.90a	4.23a
FGM	-	8.09a	7.36a	5.50a	6.20b	4.68b
-	0	8.15a	7.32a	5.56a	6.16a	4.52b
-	30	8.35a	7.46a	5.69a	6.06a	4.60b
-	60	8.15a	7.34a	5.40a	5.94a	4.50b
-	90	8.19a	7.35a	6.13b	6.05a	4.21a

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive).

Table A-8. Continued

[b] Duration of microwave (MW) exposure (s/kg soil) (2450 MHz, 700 watts) at -0.01 MPa matric potential. Following microwave treatment all soils were infested with an orange mutant pathogen at 200 cfu/g soil.

[c] Estimates of microorganism populations were made by sonication of roots from 3-week-old plants in sterile water for 5 min and the resulting suspensions dilution-plated on various agar media. Four replications of two roots each were used. Bacterial populations were estimated on nutrient agar and 1/10 strength tryptic soy agar; actinomycete populations were estimated on alkaline water agar; fluorescent pseudomonad populations were estimated on King's medium B with penicillin, cyclohexamide, and novobiocin added. Plates were examined under UV light for the production of diffusible fluorescent pigment. Colonies resembling pseudomonads that did not produce fluorescent pigments on selective King's medium B were counted and categorized as other pseudomonads. Fungal populations were estimated on potato dextrose agar with tergitol and chlortetracycline added.

[d] Means in columns within each section followed by the same letter are not significantly different ($\underline{P}<0.05$) according to Duncan's multiple range test. Additional statistical comparisons (ANOVA, Duncan's multiple range test) between the two soils at each microwave exposure level as well as within each soil over microwave exposures did not result in any significant differences other than those already indicated. For overall averages, the interaction between cultivar and soil type was not significant ($\underline{P}>0.05$) for any microorganism group. Linear regression analysis over microwave exposure was not significant ($\underline{P}<0.05$) except for populations of other pseudomonads and fungi in CSS soil ($b=0.008 \pm 0.003$ and 0.0082 ± 0.0038, respectively) and flourescent pseudomonads in the combined analysis ($b=0.0047 \pm 0.0023$).

Table A-9. Surface colonization of roots of two watermelon cultivars by Fusarium oxysporum in relation to soil populations and disease incidence in four soils after four successive watermelon plantings (Early experiment similar to that in Table 4-1).

Soil type[a]	Colonies per 100 cm root[b]		Colonization ratio[c]	% Wilt	Soil populations[d] (cfu/g)	
	OM pathogen	F. oxysporum			OM pathogen	F. oxysporum
'Crimson Sweet'						
CSS	12.2a[e]	55.0b	0.24a	19a	250a	2375a
FGM	26.7b	77.9b	0.39a	43b	450a	2038ab
LFC	44.4c	23.9a	2.12b	73c	840b	1338b
CSMW	-	-	-	100d	990b	133c
'Florida Giant'						
CSS	16.8	95.1	0.173	73a	400a	1294a
FGM	25.2	75.6	0.292	94ab	490a	1550a
LFC	-	-	-	100b	1690b	700b
CSMW	-	-	-	100b	1960b	25c

[a] Soil type represents differences in the ability of a soil to suppress Fusarium wilt of watermelon. CSS='Crimson Sweet' suppressive, monoculture soil; FGM='Florida Giant' monoculture soil (nonsuppressive); LFC=Leesburg fallow soil (conducive); CSMW=microwave-treated, 'Crimson Sweet' soil (conducive).

[b] Colonization of roots by the orange mutant (OM) strain of F. oxysporum f. sp. niveum and all F. oxysporum other than the OM pathogen was determined by washing the roots of 3-week-old plants and embedding them intact in Komada's (1975) medium. Four replications of four to six roots each were used. Root length was estimated by the line-intersect method (Tennant, 1975).

[c] The colonization ratio represents the mean of the colonization by the OM pathogen divided by the colonization by other F. oxysporum calculated for each sample.

[d] Soil populations of the OM pathogen and other F. oxysporum were determined by dilution-plating at the time of root colonization measurements. Initial inoculum was approximately 200 cfu/g soil.

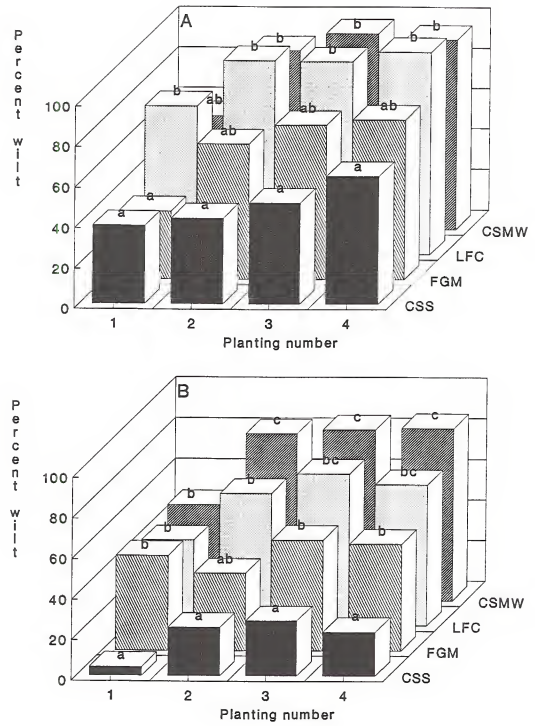
[e] Means within columns for each cultivar followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Table A-10. Effectiveness of isolates from various microorganism groups in reducing disease in initial screening tests in microwave-treated soil (Summary of results from all screening tests).

Test	Total # isolates tested	Microorganism isolates (# effective/total # tested for each microrganism group)[a]				
		F. oxysporum	Other fungi	Fluorescent pseudomonads	Other bacteria	Actinomycetes
1	28	8/10	-	1/8	1/10	-
2	22	0/3	2/4	-	3/15	-
3	19	2/2	0/5	0/2	2/10	-
4	24	2/4	0/4	-	3/16	-
5	20	2/10	-	2/4	1/6	-
6	26	5/6	0/4	1/4	4/6	2/6
7	27	6/7	-	2/7	2/10	0/3
8	38	10/19	-	1/3	5/16	-
9	34	5/13	0/1	-	7/20	-
10	23	-	3/11	-	4/12	-
11	20	6/8	-	-	2/11	0/1
12	24	3/8	0/2	1/2	2/12	-
13	18	3/8	-	-	-	3/10
14	32	11/20	0/4	0/2	0/6	-
15	40	15/22	-	1/8	-	3/10
Totals	395	78/140	5/35	9/40	36/150	8/40

[a] Values represent the numbers of isolates that were effective in significantly ($P<0.05$) reducing Fusarium wilt (relative to the pathogen-infested control) when added to microwave-treated soil followed by the total number of isolates tested for each microorganism group and individual screening test. Chlamydospore inoculum of an orange mutant pathogen was added to microwave-treated soil at 200 cfu/g soil, followed by the introduction of a conidial suspension of the potential antagonist, at 10^4 cfu/g soil for fungi and 10^5-10^7 cfu/g soil for bacteria. Five 'Crimson Sweet' watermelon seeds were planted per pot (five pots/treatment) and disease was assessed as the percentage of wilted plants after 4 weeks of growth. Disease in the pathogen-infested, control soil averaged 81 ± 7% wilt over all tests.

Figure A-1. Fusarium wilt in four soils with successive plantings of two different watermelon cultivars (Repeat of test shown in Figure 4-3). A) Watermelon cultivar 'Florida Giant' (susceptible to Fusarium wilt); B) cultivar 'Crimson Sweet' (moderately resistant and inducer of soil suppressiveness). CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment. Values within each planting topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.


A
B
Percent wilt
100
80
60
40
20
0
1
2
3
4
Planting number
CSMW
LFC
FGM
CSS

APPENDIX B
ADDITIONAL SUCCESSIVE PLANTING EXPERIMENT

Prior to the use of orange-colored mutant strains of the pathogen as markers, an experiment was conducted on the effects of successive planting of watermelon cultivars on Fusarium wilt and microorganism populations in four soils suppressive and conducive to the disease. The experiment was conducted using four different isolates of the pathogen, which represented the two races and three VCGs established in Fusarium oxysporum f. sp. niveum.

The experiment utilized a three-way factorial design, using four watermelon cultivars ('Florida Giant,' susceptible to Fusarium wilt; 'Charleston Gray' and 'Crimson Sweet,' moderately resistant; 'Calhoun Gray,' highly resistant), four pathogen isolates from Florida (FG85-1, VCG 0080, race 1; FG85-2 and FG85-15, VCG 0081, race 1; and CS85-4, VCG 0082, race 2), and four soils ('Crimson Sweet' suppressive, monoculture soil; 'Florida Giant' nonsuppressive, monoculture soil; Leesburg fallow, conducive soil; and suppressive soil rendered conducive by microwave treatment). In addition, an unplanted control for each soil was maintained for comparison of microorganism populations with the various cultivars.

Methodology was identical to that described in Chapter 4, except that the pathogen inoculum was added as conidial suspensions of 2×10^3 cfu/g soil for each pathogen isolate. Four replicate pots of 10 plants each were planted with each cultivar for each pathogen and soil combination. Incidence of Fusarium wilt was monitored over

4-week periods, after which plants were removed, the soil stirred, and each pot was replanted with the same cultivar as before. This cycle was repeated through five successive plantings. After the fourth planting, soil microorganism populations were assayed as described in Chapter 3.

Because no marked pathogen strains were used in these tests, it was impossible to determine precise pathogen populations in these soils. Since all of the pathogens that were added to these soils produced a red pigment when cultured on PDA or Komada's (1975) medium, population estimates of red pigmented F. oxysporum organisms were assayed. These estimates included the pathogens, but also included some indigenous and nonpathogenic isolates of F. oxysporum. However, since many indigenous isolates do not produce this pigment and the pathogen was added at levels greater than the natural populations, a large proportion of the population of pigmented organisms was composed of the introduced pathogen. Thus, although not a true measure of the pathogen inoculum dynamics, this did provide a better estimate of possible changes in pathogen populations than the total population of all F. oxysporum would have.

Some results from this test (cultivars 'Florida Giant' and 'Crimson Sweet') were included in Chapter 4. Complete results are summarized in the figures on the following pages. Data on the development of Fusarium wilt, beginning with overall cultivar and soil averages and followed by individual results for each treatment combination, are contained in Figures B-1 to B-6. Microorganism population estimates are depicted in Figures B-7 to B-9. Average populations of F. oxysporum are shown in Figure B-10. All population estimates were averaged over all four pathogen isolates. There were no significant ($P<0.05$) effects due to different pathogen isolates on other microorganism populations.

Figure B-1. Fusarium wilt development in four soils with successive plantings of four different watermelon cultivars (Comparison by soil type for each cultivar; average over all pathogen isolates). A) Watermelon cultivar 'Florida Giant' (susceptible to Fusarium wilt); B) cultivar 'Charleston Gray' (moderately resistant); C) cultivar 'Crimson Sweet' (moderately resistant and inducer of soil suppressiveness); D) cultivar 'Calhoun Gray' (highly resistant). CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment.

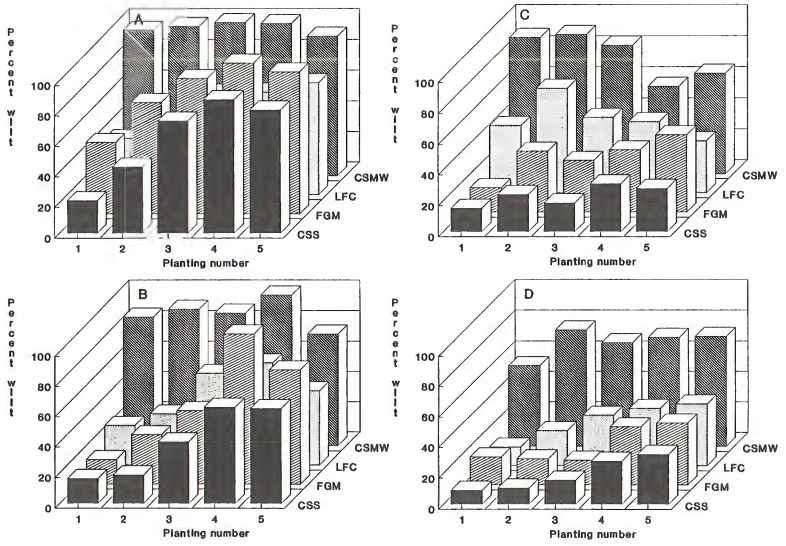

A
B
C
D
Percent wilt
0
20
40
60
80
100
1
2
3
4
5
Planting number
CSS
FGM
LFC
CSMW

Figure B-2. Fusarium wilt development in four soils with successive plantings of four different watermelon cultivars (Comparison by cultivar for each soil; average over all pathogen isolates). A) CSS (suppressive, monoculture) soil; B) FGM (nonsuppressive, monoculture) soil; C) LFC (fallow, conducive) soil; D) CSMW soil (suppressive soil rendered conducive by microwave treatment). Watermelon cultivars: FG='Florida Giant' (susceptible to Fusarium wilt), CHG='Charleston Gray' (moderately resistant), CS='Crimson Sweet' (moderately resistant and inducer of soil suppressiveness), and CLG='Calhoun Gray' (highly resistant).

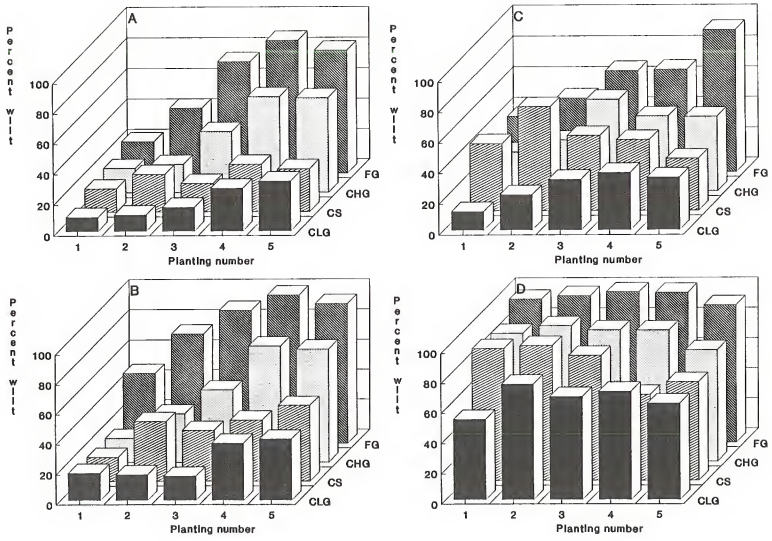

A
B
C
D
Percent wilt
100
80
60
40
20
0
1
2
3
4
5
Planting number
FG
CHG
CS
CLG

Figure B-3. Fusarium wilt development in four soils with successive plantings of watermelon cultivar 'Florida Giant' (susceptible to Fusarium wilt) for four different isolates of Fusarium oxysporum f. sp. niveum. A) pathogen isolate FG85-1 (VCG 0080, race 1); B) isolate FG85-2 (VCG 0081, race 1); C) isolate FG85-15 (VCG 0081, race 1); D) isolate CS85-4 (VCG 0082, race 2). CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment.

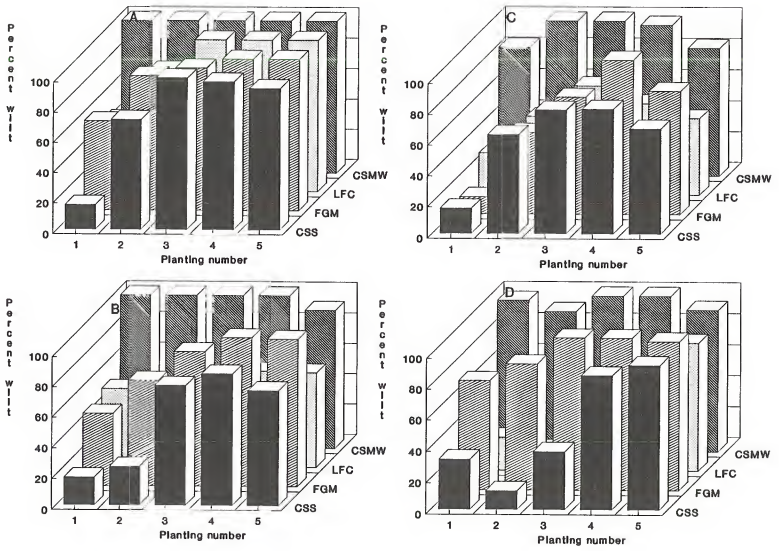

A
B
C
D
Percent wilt
0
20
40
60
80
100
1
2
3
4
5
Planting number
CSS
FGM
LFC
CSMW

Figure B-4. Fusarium wilt development in four soils with successive plantings of watermelon cultivar 'Charleston Gray' (moderately resistant to Fusarium wilt) for four different isolates of Fusarium oxysporum f. sp. niveum. A) pathogen isolate FG85-1 (VCG 0080, race 1); B) isolate FG85-2 (VCG 0081, race 1); C) isolate FG85-15 (VCG 0081, race 1); D) isolate CS85-4 (VCG 0082, race 2). CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment.

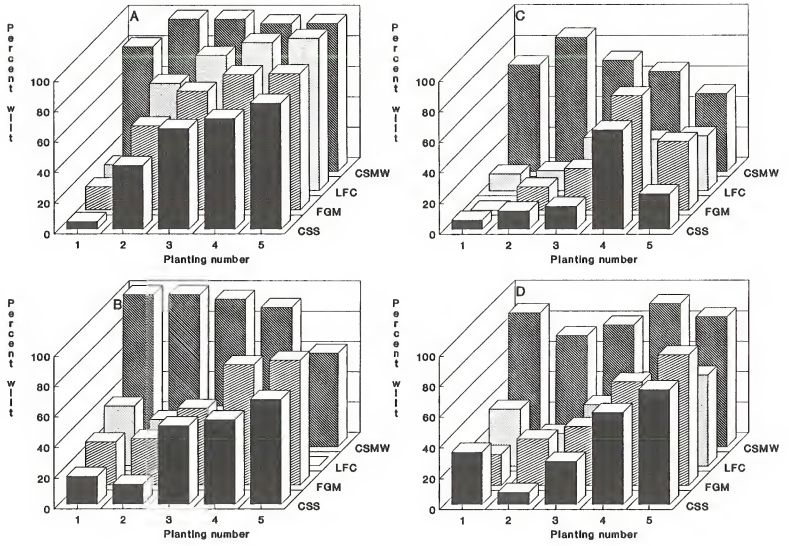

A
B
C
D
Percent wilt
0
20
40
60
80
100
1
2
3
4
5
Planting number
CSS
FGM
LFC
CSMW

Figure B-5. Fusarium wilt development in four soils with successive plantings of watermelon cultivar 'Crimson Sweet' (moderately resistant to Fusarium wilt and inducer of soil suppressiveness) for four different isolates of Fusarium oxysporum f. sp. niveum. A) pathogen isolate FG85-1 (VCG 0080, race 1); B) isolate FG85-2 (VCG 0081, race 1); C) isolate FG85-15 (VCG 0081, race 1); D) isolate CS85-4 (VCG 0082, race 2). CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment.

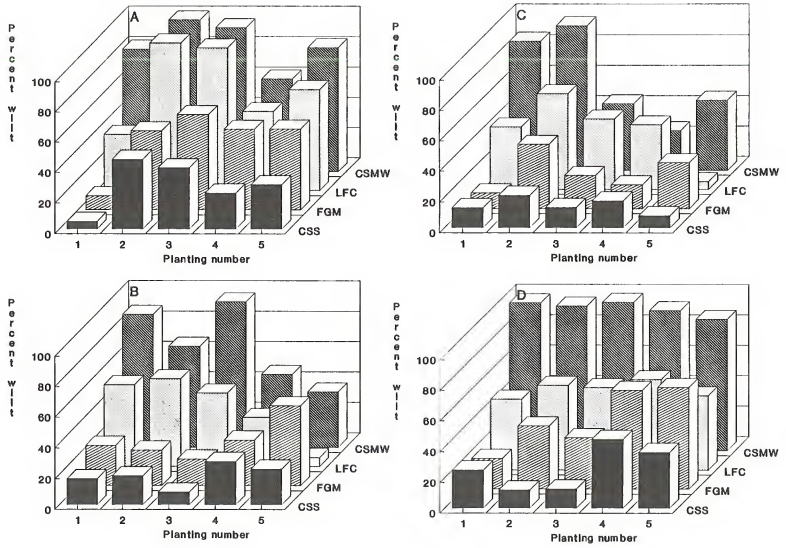

A
B
C
D
Percent wilt
0
20
40
60
80
100
1
2
3
4
5
Planting number
CSS
FGM
LFC
CSMW

Figure B-6. Fusarium wilt development in four soils with successive plantings of watermelon cultivar 'Calhoun Gray' (highly resistant to Fusarium wilt) for four different isolates of Fusarium oxysporum f. sp. niveum. A) pathogen isolate FG85-1 (VCG 0080, race 1); B) isolate FG85-2 (VCG 0081, race 1); C) isolate FG85-15 (VCG 0081, race 1); D) isolate CS85-4 (VCG 0082, race 2). CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment.

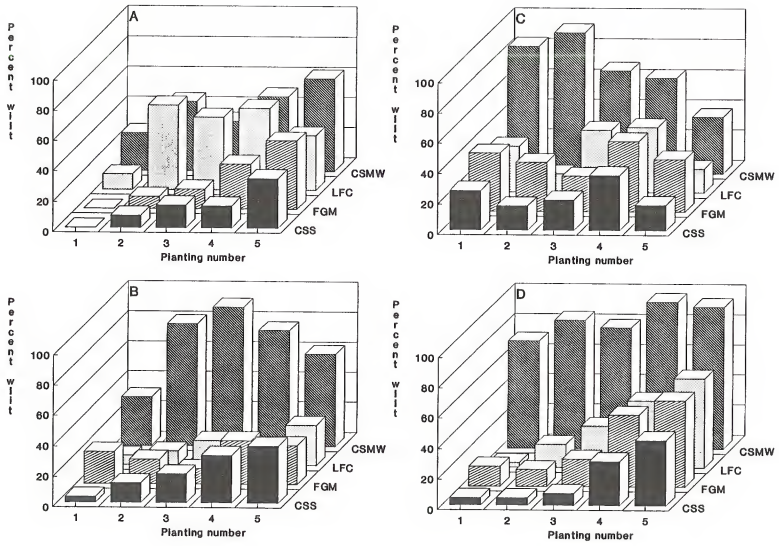
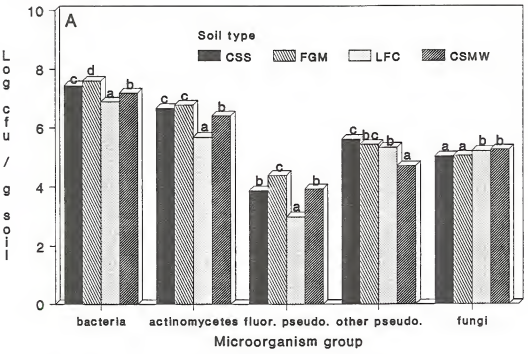

A
B
C
D
Percent wilt
0
20
40
60
80
100
1
2
3
4
5
Planting number
CSS
FGM
LFC
CSMW

Figure B-7. Average populations of bacteria, actinomycetes, fluorescent pseudomonads, other pseudomonads, and fungi in four soils after four successive plantings of various watermelon cultivars. A) Average populations by soil type over all cultivars (CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment); B) Average populations by watermelon cultivar over all soil types (Watermelon cultivars: FG='Florida Giant,' susceptible to Fusarium wilt; CHG='Charleston Gray,' moderately resistant; CS='Crimson Sweet,' moderately resistant and inducer of soil suppressiveness; and CLG='Calhoun Gray,' highly resistant). Values within each microorganism group topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.


A
Soil type
CSS
FGM
LFC
CSMW
Log cfu / g soil
10
8
6
4
2
0
bacteria
actinomycetes
fluor. pseudo.
other pseudo.
fungi
Microorganism group

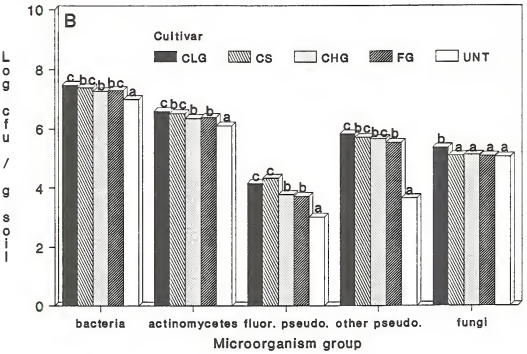

B
Cultivar
CLG
CS
CHG
FG
UNT
Log cfu / g soil
10
8
6
4
2
0
bacteria
actinomycetes
fluor. pseudo.
other pseudo.
fungi
Microorganism group

Figure B-8. Populations of bacteria, actinomycetes, fluorescent pseudomonads, other pseudomonads, and fungi in four soils after four successive plantings of various watermelon cultivars (Comparison by soil type for each cultivar). A) Watermelon cultivar 'Calhoun Gray' (highly resistant to Fusarium wilt of watermelon); B) cultivar 'Charleston Gray' (moderately resistant); C) cultivar 'Crimson Sweet' (moderately resistant and inducer of soil suppressiveness); D) cultivar 'Florida Giant' (susceptible). CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment. Values within each microorganism group topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

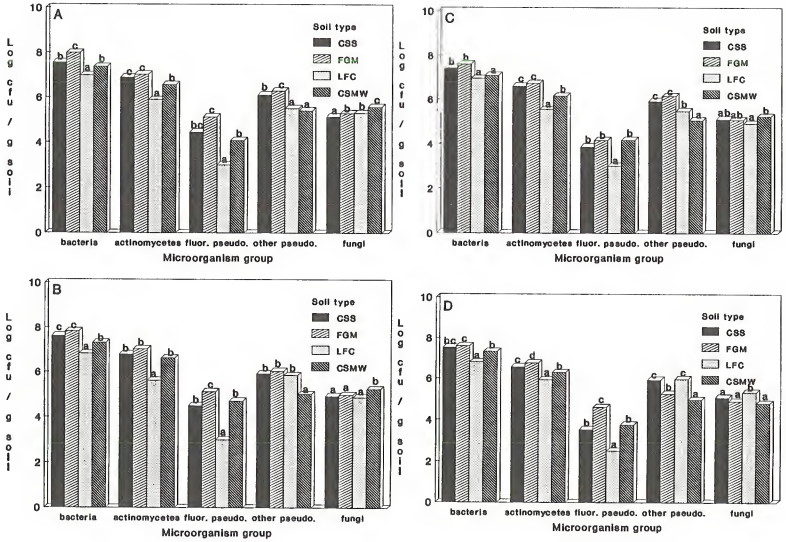

A
B
C
D
Soil type
CSS
FGM
LFC
CSMW
Log cfu / g soil
bacteria
actinomycetes
fluor. pseudo.
other pseudo.
fungi
Microorganism group

Figure B-9. Populations of bacteria, actinomycetes, fluorescent pseudomonads, other pseudomonads, and fungi in four soils after four successive plantings of various watermelon cultivars (Comparison by cultivar for each soil type). A) CSS (suppressive, monoculture) soil; B) FGM (nonsuppressive, monoculture) soil; C) LFC (fallow, conducive) soil; D) CSMW soil (suppressive soil rendered conducive by microwave treatment). Watermelon cultivars: FG='Florida Giant' (susceptible to Fusarium wilt), CHG='Charleston Gray' (moderately resistant), CS='Crimson Sweet' (moderately resistant and inducer of soil suppressiveness), CLG='Calhoun Gray' (highly resistant), and UNT=control soil remaining unplanted. Values within each microorganism group topped by the same letter are not significantly different ($\underline{P}<0.05$) according to Duncan's multiple range test.

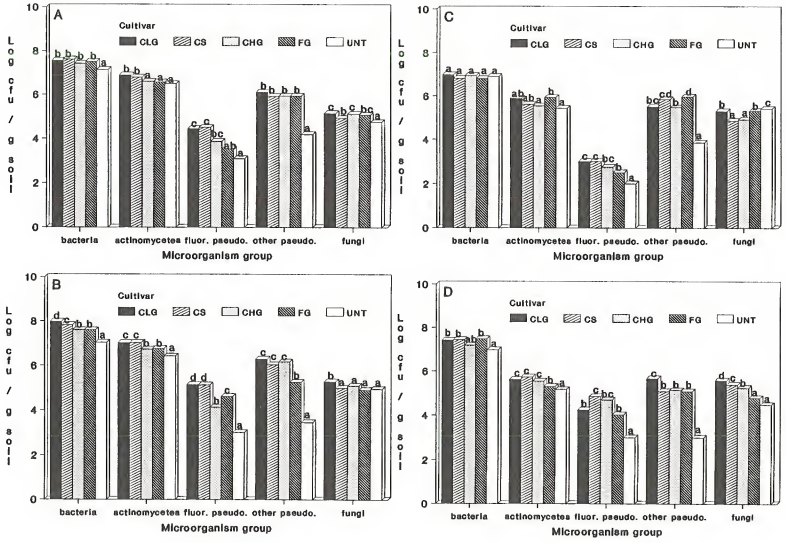
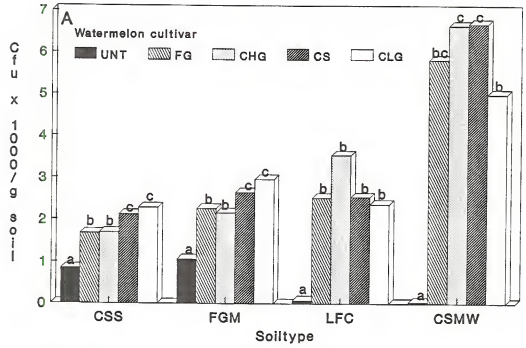
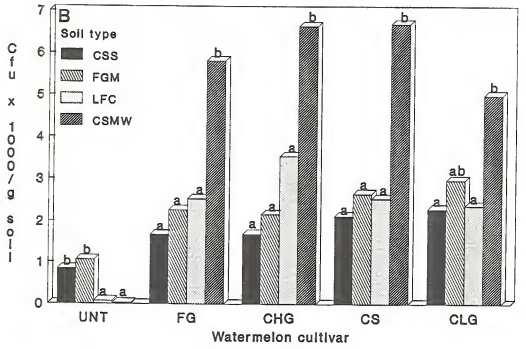

A
B
C
D
Cultivar
CLG
CS
CHG
FG
UNT
Log cfu / g soil
bacteria
actinomycetes
fluor. pseudo.
other pseudo.
fungi
Microorganism group

Figure B-10. Population estimates of red-pigmented isolates of Fusarium oxysporum in four soils after four successive plantings of various watermelon cultivars. A) Average populations compared by watermelon cultivar within each soil type; B) Average populations compared by soil type for each watermelon cultivar (Watermelon cultivars: FG='Florida Giant' (susceptible to Fusarium wilt), CHG='Charleston Gray' (moderately resistant), CS='Crimson Sweet' (moderately resistant and inducer of soil suppressiveness), CLG='Calhoun Gray' (highly resistant), and UNT=control soil remaining unplanted. Soil types: CSS=suppressive, monoculture soil; FGM=nonsuppressive, monoculture soil; LFC=fallow, conducive soil; CSMW=suppressive soil rendered conducive by microwave treatment). Values within each cluster topped by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.


A
Watermelon cultivar
UNT
FG
CHG
CS
CLG
Cfu x 1000/g soil
0
1
2
3
4
5
6
7
a
b
b
c
a
b
b
c
c
a
b
b
b
b
a
bc
c
c
b
CSS
FGM
LFC
CSMW
Soiltype


B
Soil type
CSS
FGM
LFC
CSMW
Cfu x 1000/g soil
0
1
2
3
4
5
6
7
b
b
a
a
a
a
a
b
a
a
a
b
a
a
a
b
a
ab
a
b
UNT
FG
CHG
CS
CLG
Watermelon cultivar

LITERATURE CITED

Adams, P.B. 1967. A buried membrane filter method for studying behavior of soil fungi. Phytopathology 57:602-603.

Alabouvette, C. 1986. Fusarium-wilt suppressive soils from the Chateaurenard region: Review of a 10-year study. Agronomie 6:273-284.

Alabouvette, C. 1989. Manipulation of soil environment to create suppressiveness in soils. In: Vascular Wilt Diseases of Plants, Tjamos, E.C., and Beckman, C.H., eds., pp. 457-478. Springer-Verlag, NY. 590 pp.

Alabouvette, C., Couteaudier, Y., and Louvet, J. 1985a. Recherches sur la résistance des sols aux maladies. XI. Etude comparative du comportement des Fusarium spp. dans un sol résistant et un sol sensible aux fusarioses vasculaires enrichis en glucose. Agronomie 5:63-68.

Alabouvette, C., Couteaudier, Y., and Louvet, J. 1985b. Soils suppressive to Fusarium wilt: Mechanisms and management of suppressiveness. In: Ecology and Management of Soil-Borne Plant Pathogens, Parker, C.A., Moore, D.J., Wong, P.T.W., Rovira, A.D., and Kollmorgen, J.F., eds., pp. 101-106. American Phytopathological Society, St.Paul, MN. 358 pp.

Alabouvette, C., Rouxel, F., and Louvet, J. 1979. Characteristics of Fusarium wilt-suppressive soils and prospects for their utilization in biological control. In: Soil-Borne Plant Pathogens, Schippers, B. and Gams, W., eds., pp. 165-182. Academic Press, New York 686 pp.

Alabouvette, C., Rouxel, F., and Louvet, J. 1980. Recherches sur la résistance des sols aux maladies. VII. Etude comparative de la germination des chlamydospores de Fusarium oxysporum et Fusarium solani au contact de sol résistant et sensible aux fusarioses vasculaires. Ann. Phytopathol. 12:21-30.

Armstrong, G.M., and Armstrong, J.K. 1948. Nonsusceptible hosts as carriers of wilt fusaria. Phytopathology 38:808-826.

Armstrong, G.M., and Armstrong, J.K. 1978. Formae speciales and races of Fusarium oxysporum causing wilts of the cucurbitaceae. Phytopathology 68:19-28.

Atkinson, T.G., Neal, J.L., Jr., and Larson, R.L. Genetic control of the rhizosphere microflora of wheat. In: Biology and Control of Soil-Borne Plant Pathogens, Bruehl, G.W., ed., pp. 116-122. American Phytopathological Society, St. Paul, MN. 216 pp.

Baker, K.F., and Cook, R.J. 1974. Biological Control of Plant Pathogens. W.H. Freeman, San Francisco. 433 pp.

Baker, R., and Chet, I. 1982. Induction of suppressiveness. In: Suppressive Soils and Plant Disease, Schneider, R.W., ed., pp. 35-50. American Phytopathological Society, St. Paul, MN. 216 pp.

Banihashemi, Z., and deZeeuw, D.J. 1975. The behavior of Fusarium oxysporum f. sp. melonis in the presence and absence of host plants. Phytopathology 65:1212-1217.

Beckman, C.H. 1987. The Nature of Wilt Diseases of Plants. American Phytopathological Society, St. Paul, MN. 175 pp.

Beckman, C.H., and Halmos, S. 1962. Relation of vascular occluding reactions in banana plants to pathogenicity of root-invading fungi. Phytopathology 52:699-702.

Beckman, C.H., Mueller, W.C., Tessier, B.J., and Harrison, N.A. 1982. Recognition and callose deposition in response to vascular infection in Fusarium wilt-resistant or susceptible tomato plants. Physiol. Plant Pathol. 20:1-10.

Biles, C.L., and Martyn, R.D. 1989. Local and systemic resistance induced in watermelon by formae speciales of Fusarium oxysporum. Phytopathology 79:856-860.

Bosland, P.W., and Williams, P.H. 1987. An evaluation of Fusarium oxysporum from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility and geographical origin. Can. J. Bot. 65:2067-2073.

Bosland, P.W., Williams, P.H., and Morrison, R.H. 1988. Influence of soil temperature on expression of yellows and wilt of crucifers by Fusarium oxysporum. Plant Disease 72:777-778.

Bourbos, V.A. and Skoudridakis, M.T. 1987. Das verhalten einiger pilzlicher antagonisten in der rhizosphäre resisenter und anfälliger gewächshaustomaten. J. Phytopathology 120:193-198.

Bruton, B.D., Patterson, C.L., and Martyn, R.D. 1988. Fusarium wilt (F. oxysporum f. sp. niveum Race 2) of watermelon in Oklahoma. (Abstr.) Plant Disease 72:734.

Buxton, E.W. 1975. Differential rhizosphere effect of three pea cultivars on physiological races of Fusarium oxysporum f. sp. pisi. Trans. Brit. Mycol. Soc. 40:145-154.

Caten, C.E. 1987. The concept of race in plant pathology. In: Populations of Plant Pathogens, Wolfe, M.S, and Caten, C.E., eds., pp. 35-56. Blackwell Scientific Publications, London. 350 pp.

Chet, I., and Baker, R. 1980. Induction of suppressiveness to Rhizoctonia solani in soil. Phytopathology 70:994-998.

Cirulli, M. 1972. Variation of pathogenicity in Fusarium oxysporum f. sp.niveum and resistance in watermelon cultivars. Actas Congr. Uniao. Fitopatol. Mediterr. 3rd. pp. 491-500.

Cook, R.J. 1981. The influence of rotation crops on take-all decline phenomenon. Phytopathology 71:189-192.

Cook, R.J., and Baker, K.F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St.Paul, MN. 433 pp.

Cook, R.J., and Rovira, A.D. 1976. The role of bacteria in the biological control of Gaeumannomyces graminis by suppressive soils. Soil Biol. Biochem. 8:267-273.

Correll, J.C., Klittich, C.J.R., and Leslie, J.F. 1987. Nitrate nonutilizing mutants of Fusarium oxysporum and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.

Correll, J.C., Puhalla, J.E., and Schneider, R.W. 1986a. Identification of Fusarium oxysporum f. sp. apii on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76:396-400.

Correll, J.C., Puhalla, J.E., and Schneider, R.W. 1986b. Vegetative compatibility groups among nonpathogenic root colonizing strains of Fusarium oxysporum. Can. J. Bot. 64:2358-2361.

Correll, J.C., Puhalla, J.E., Schneider, R.W., and Kraft, J.M. 1985. Differentiating races of Fusarium oxysporum f. sp. pisi based on vegetative compatibility. (Abstr.) Phytopathology 75:1347.

Crall, J.M. 1963. Physiological specialization in Fusarium oxysporum f. sp. niveum. (Abstr.) Phytopathology 53:873.

Davis, D. 1967. Cross-protection in Fusarium wilt diseases. Phytopathology 57:311-314.

Davis, D. 1968. Partial control of Fusarium wilt in tomato by formae of Fusarium oxysporum. Phytopathology 58:121-122.

Dean, R.A., and Kuc, J. 1986. Induced systemic protection in cucumber: Time of production and movement of signal. Phytopathology 76:966-970.

Elad, Y., and Baker, R. 1985a. Influence of trace amounts of cations and siderophore-producing pseudomonads on chlamydospore germination of Fusarium oxysporum. Phytopathology 75:1047-1052.

Elad, Y., and Baker, R. 1985b. The role of competition of iron and carbon in suppression of chlamydospore germination of Fusarium spp. by Pseudomonas spp. Phytopathology 75:1053-1059.

Elias, K.S., and Schneider, R.W. 1987. Origin and relatedness of races within Fusarium oxysporum f. sp. lycopersici and nonpathogenic strains of F. oxysporum. (Abstr.) Phytopathology 77:1710.

Elmer, W.H., and Lacy, M.L. 1987. Effects of crop residues and colonization of plant tissues on propagule survival and soil populations of Fusarium oxysporum f. sp. asparagi race 2. Phytopathology 77:381-387.

Elmer, W.H., and Stephens, C.T. 1989. Classification of Fusarium oxysporum f. sp. asparagi into vegetatively compatible groups. Phytopathology 79:88-93.

Elmstrom, G.W., and Hopkins, D.L. 1981. Resistance of watermelon cultivars to Fusarium wilt. Plant Disease 65:825-827.

English, J.T., and Mitchell, D.J. 1988. Development of microbial communities associated with tobacco root systems. Soil Biol. Biochem. 20:137-144.

Ferriss, R.S. 1984. Effects of microwave oven treatment on microorganisms in soil. Phytopathology 74:121-126.

Foster, R.C., and Bowen, G.D. 1982. Plant surfaces and bacterial growth: The rhizosphere and rhizoplane. In: Phytopathogenic Prokaryotes, vol. 1, Mount, M.S., and Lacy, G.H., eds., pp. 159-185. Academic Press, NY. 465 pp.

Gerlagh, M. 1968. Introduction of Ophiobolus graminis into new polders and its decline. Neth. J. Plant Pathol. Suppl. 2:1-97.

Gessler, C., and Kuc, J. 1982. Induction of resistance to Fusarium wilt of cucumber by root and foliar pathogens. Phytopathology 73:1439-1441.

Gordon, T.R., and Okamoto, D. 1990. Colonization of crop residue by Fusarium oxysporum f. sp. melonis and other species of Fusarium. Phytopathology 80:381-386.

Gordon, T.R., Okamoto, D., and Jacobsen, D.J. 1989. Colonization of muskmelon and nonsusceptible crops by Fusarium oxysporum f. sp. melonis and other species of Fusarium. Phytopathology 79:1095-1100.

Hamdan, H., Thomashow, L.S., and Weller, D.M. 1988. Relative importance of fluorescent siderophore and phenazine antibiotic by Pseudomonas fluorescens 2-79 in suppression of take-all. (Abstr.) Phytopathology 78:1522.

Henderson, W.R., Jenkins, S.F., and Rawlings, J.O. 1970. The inheritance of Fusarium wilt resistance in watermelon, Citrullus lanatus (Thunb.) Mansf. J. Amer. Soc. Hort. Sci. 95:276-282.

Hendrix, F.F., and Nielsen, L.W. 1958. Invasion and infection of crops other than the forma suscept by Fusarium oxysporum f. sp. batata and other formae. Phytopathology 48:224-228.

Henis, Y., Ghaffar, A., and Baker, R. 1978. Integrated control of Rhizoctonia solani damping-off of radish: Effect of successive plantings, PCNB, and Trichoderma harzianum on pathogen and disease. Phytopathology 68:900-907.

Henis, Y., Ghaffar, A., and Baker, R. 1979. Factors affecting suppressiveness to Rhizoctonia solani in soil. Phytopathology 69:1164-1169.

Hillocks, R.J. 1986. Cross protection between strains of Fusarium oxysporum f. sp. vasinfectum and its effect on vascular resistance mechanisms. J. Phytopathology 117:216-225.

Ho, W.C, and Ko, W.H. 1980. A simple medium for selective isolation and enumeration of soil actinomycetes. Ann. Phytopathol. Soc. Japan 46:634-638.

Ho, W.C, and Ko, W.H. 1982. Characteristics of soil microbiostasis. Soil Biol. Biochem. 14:589-593.

Hopkins, D.L. 1985. Monoculture of Crimson Sweet watermelon promotes soil suppressiveness to Fusarium wilt. (Abstr.) Phytopathology 75:1343-1344.

Hopkins, D.L., and Elmstrom, G.W. 1984. Fusarium wilt in watermelon cultivars grown in a 4-year monoculture. Plant Disease 68:239-241.

Hopkins, D.L., Larkin, R.P., and Elmstrom, G.W. 1987. Cultivar-specific induction of soil suppressiveness to Fusarium wilt of watermelon. Phytopathology 77:607-611.

Hopkins, D.L., Larkin, R.P., and Lobinske, R.J. 1989. Effect of watermelon cultivar monoculture on aggressiveness of Fusarium oxysporum f. sp. niveum. (Abstr.) Phytopathology 78:1542.

Hopkins, D.L., and Lobinske, R.J. 1990. Use of a highly susceptible watermelon cultivar to selectively recover Fusarium oxysporum f. sp. niveum from soil for race determination. (Abstr.) Phytopathology 80:1009.

Hornby, D. 1983. Suppressive soils. Ann. Rev. Phytopathol. 21:65-85.

Huang, J.W., Sun, S.K., and Ko, W.H. 1988. Characteristics of inhibition of a Taiwanese soil suppressive to Fusarium oxysporum f. sp. raphani. J. Phytopathology 123:165-173.

Hwang, S.F., Cook, R.J., and Haglund, W.A. 1982. Mechanisms of suppression of chlamydospore germination for Fusarium oxysporum f. sp. pisi in soils. (Abstr.) Phytopathology 72:948.

Ireland, K.F., and Lacy, M.L. 1986. Laboratory identification of pathogenic isolates of Fusarium oxysporum f. sp. apii race 2. (Abstr.) Phytopathology 76:956.

Jacobsen, D.J., and Gordon, T.R. 1988. Vegetative compatibility and self-incompatibility within Fusarium oxysporum f. sp. melonis. Phytopathology 78:668-672.

Katan, J. 1971. Symptomless carriers of the tomato Fusarium wilt pathogen. Phytopathology 61:1213-1217.

Katan, J., Fishler, G., and Grinstein, A. 1983. Short- and long-term effects of soil solarization on Fusarium wilt and yield of cotton in Israel. Phytopathology 73:1215-1219.

Katan, T., Hadar, E., and Katan, J. 1989. Vegetaive compatibility of Fusarium oxysporum f. sp. dianthi from carnation in Israel. Plant Pathol. 38: 376-381.

Katan, T., and Katan, J. 1988. Vegetative-compatibility grouping of Fusarium oxysporum f. sp. vasinfectum from tissue and the rhizosphere of cotton plants. Phytopathology 78:852-855.

Kloepper, J.W., Leong, J., Teintze, M., and Schroth, M.N. 1980. Pseudomonas siderophores: A mechanism explaining disease-suppressive soils. Curr. Microbiol. 4:317-320.

Kloepper, J.W., and Schroth, M.N. 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathology 71:1020-1024.

Komada, H. 1975. Development of a selective medium for quantitative isolation of Fusarium oxysporum from natural soil. Rev. Plant Prot. Res. 8:115-125.

Kommedahl, T., Christensen, J.J., and Frederickson, R.A. 1970. A half century of research in Minnesota on flax wilt caused by Fusarium oxysporum. Minn. Agric. Exp. Stn. Tech. Bull. No. 273. 35 pp.

Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1988. Differentiation of strains and pathogenic races of Fusarium oxysporum f. sp. niveum based on vegetative compatibility. (Abstr.) Phytopathology 88:1542.

Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1989. Ecology of Fusarium oxysporum f. sp. niveum in Fusarium wilt-suppressive and -conducive soils. (Abstr.) Phytopathology 79:1166.

Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1990. The roles of indigenous Fusarium oxysporum and various other microorganisms in a soil suppressive to Fusarium wilt of watermelon. (Abstr.) Phytopathology 80:995-996.

Latin, R.X., and Snell, S.J. 1986. Comparison of methods for inoculation of muskmelon with Fusarium oxysporum f. sp. melonis. Plant Disease 70:287-300.

Lemanceau, P. 1989. Role of competition for carbon and iron in mechanisms of soil suppressiveness to Fusarium wilts. In: Vascular Wilt Diseases of Plants, Tjamos, E.C., and Beckman, C.H., eds., pp. 385-396. Springer-Verlag, NY. 590 pp.

Lemanceau, P., Alabouvette, C., and Couteaudier, Y. 1988. Recherches sur la résistance des sols aux maladies XIV. Modification du niveau de réceptivité d'un sol résistant et d'un sol sensible aux fusarioses vasculaires en réponse a des apports de fer ou de glucose. Agronomie 8:155-162.

Lin, Y.S., and Cook, R.J. 1979. Suppression of Fusarium roseum 'Avenaceum' by soil microorganisms. Phytopathology 69:384-388.

Liu, S., and Baker, R. 1980. Mechanism of biological control in soil suppressive to Rhizoctonia solani. Phytopathology 70:406:412.

Locke, J.C., Marois, J.J., and Papavizas, G.C. 1985. Biological control of Fusarium wilt of greenhouse grown chrysanthemums. Plant Disease 69: 167-169.

Loper, J.E., Suslow, T.V., and Schroth, M.N. 1984. Lognormal distribution of bacterial populations in the rhizosphere. Phytopathology 74:1454-1460.

Louvet, J. 1989. Microbial populations and mechanisms determining soil suppressiveness to Fusarium wilts. In: Vascular Wilt Diseases of Plants, Tjamos, E.C., and Beckman, C.H., eds., pp. 367-384. Springer-Verlag, NY. 590 pp.

Louvet, J., Alabouvette, C., and Rouxel, F. 1981. Microbiological suppressiveness of some soils to Fusarium wilts. In: Fusarium: Diseases, Biology, and Taxonomy. Nelson, P.E., Toussoun, T.A.,and Cook, R.J., eds., pp. 261-275. Pennsylvania State University Press, University Park, PA. 457 pp.

MacHardy, W.E., and Beckman, C.H. 1981. Vascular wilt Fusaria: Infection and pathogenesis. In: Fusarium: Diseases, Biology, and Taxonomy, Nelson, P.E., Toussoun, T.A., and Cook, R.J., eds., pp. 365-390. Pennsylvania State University Press, University Park, PA. 475pp.

Mall, S. 1979. Rhizosphere and rhizoplane microflora of three potato varieties. Indian Phytopathology 27:347-354.

Maraite, H. 1982. Induced resistance in muskmelon wilt. In: Active Defense Mechanisms in Plants, Wood, R.K.S., ed., pp. 339-340. Plenum Press, NY. 381pp.

Marois, J.J., Mitchell, D.J., and Sonoda, R.M. 1981. Biological control of Fusarium crown rot of tomato under field conditions. Phytopathology 71:1257-1260.

Martyn, R.D. 1987. Fusarium oxysporum f. sp. niveum race 2: A highly aggressive race new to the United States. Plant Disease 71:233-236.

Martyn, R.D., Dillard, E.A., and Biles, C.L. 1990. Effects of challenge inoculum on induced resistance to Fusarium wilt of watermelon under simulated field conditions. (Abstr.) Phytopathology 80:436-437.

Martyn, R.D., and McLaughlin, R.J. 1983. Effects of inoculum concentration on the apparent resistance of watermelons to Fusarium oxysporum f. sp. niveum. Plant Disease 67:493-495.

Matta, A. 1989. Induced resistance to Fusarium wilt diseases. In: Vascular Wilt Diseases of Plants, Tjamos, E.C., and Beckman, C.H., eds., pp. 175-195. Springer-Verlag, NY. 590 pp.

McKeen, C.D. 1951. Investigation of Fusarium wilt of muskmelons and watermelons in Southwestern Ontario. Sci. Agri. 31:413-423.

Neal, J.L., Larson, R.L., and Atkinson, T.G. 1973. Changes in rhizosphere populations of selected physiological groups of bacteria related to substitution of specific pairs of chromosomes in spring wheat. Plant and Soil 39:209-212.

Nelson, P.E. 1981. Life cycle and epidemiology of Fusarium oxysporum. In: Fungal Wilt Diseases of Plants, Mace, M.E., Bell, A.A., and Beckman, C.H., eds., pp. 51-80. Academic Press, NY. 640 pp.

Netzer, D. 1976. Physiological races and soil population level of Fusarium wilt of watermelon. Phytoparasitica 4:131-136.

Netzer, D., and Weintall, C.H. 1980. Inheritance of resistance in watermelon to race 1 of Fusarium oxysporum f. sp. niveum. Plant Disease 64:853-854.

Ogawa, K., and Komada, H. 1984. Biological control of Fusarium wilt of sweet potato by non-pathogenic Fusarium oxysporum. Ann. Phytopath. Soc. Japan 50:1-9.

Ogawa, K., and Komada, H. 1985. Biological control of Fusarium wilt of sweet potato with cross-protection by nonpathogenic Fusarium oxysporum. In: Ecology and Management of Soil-Borne Plant Pathogens. Parker, C.A., Moore, D.J., Wong, P.T.W., Rovira, A.D., and Kollmorgen, J.F., eds., pp. 121-123. American Phytopathological Society, St.Paul, MN. 358 pp.

Ogawa, K., and Komada, H. 1986. Induction of systemic resistance against Fusarium wilt of sweet potato by non-pathogenic Fusarium oxysporum. Ann. Phytopath. Soc. Japan 52:15-21.

Park, C.S., Paulitz, T.C., and Baker, R. 1988. Biocontrol of Fusarium wilt of cucumber resulting from interactions between Pseudomonas putida and nonpathogenic isolates of Fusarium oxysporum. Phytopathology 78:190-194.

Paulitz, T.C., Park, C.S., and Baker, R. 1987. Biological control of Fusarium wilt of cucumber with nonpathogenic isolates of Fusarium oxysporum. Can. J. Microbiol. 33:349-353.

Pierson, L.S., and Thomashow, L.S. 1988. Role of phenazine antibiotics produced by Pseudomonas fluorescens 30-84 in take-all suppression. (Abstr.) Phytopathology 78:1522.

Ploetz, R.C., and Correll, J.C. 1988. Vegetative compatibility among races of Fusarium oxysporum f. sp. cubense. Plant Disease 72:325-328.

Puhalla, J.E. 1984. A visual indicator of heterokaryosis in Fusarium oxysporum from celery. Can. J. Bot. 62:540-545.

Puhalla, J.E. 1985. Classification of strains of Fusarium oxysporum on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.

Reid, J. 1958. Studies on the fusaria which cause wilt in melons. 1. The occurrence and distribution of races of the muskmelon and watermelon fusaria and a histological study of the colonization of muskmelon plants susceptible or resistant to Fusarium wilt. Can. J. Bot. 36:393-410.

Reyes, A.A., and Mitchell, J.E. 1962. Growth response of several isolates of Fusarium in rhizospheres of host and nonhost plants. Phytopathology 52:1196-1200.

Rouxel, F., Alabouvette, C., and Masson, J.P. 1979. Recherches sur la résistance des sols aux maladies. IV. Mise en évidence du rôle des Fusarium autochtones dans la résistance d'un sol a la fusariose vasculaire du melon. Ann Phytopathol. 11:199-207.

Sands, D.C., and Rovira, A.D. 1970. Isolation of fluorescent pseudomonads with a selective medium. Appl. Microbiol. 20:513-514.

Scher, F.M., and Baker, R. 1980. Mechanism of biological control in a Fusarium-suppressive soil. Phytopathology 70:412-417.

Scher, F.M., and Baker, R. 1982. Effect of Pseudomonas putida and a synthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. Phytopathology 72:1567-1573.

Schneider, R.W., ed. 1982. Suppressive Soils and Plant Disease. American Phytopathology Society, St.Paul, MN. 88 pp.

Schneider, R.W. 1984. Effects of nonpathogenic strains of Fusarium oxysporum on celery root infection by Fusarium oxysporum f. sp. apii and a novel use of the Lineweaver-Burk double reciprocal plot technique. Phytopathology 74:646-653.

Schroth, M.N., and Hancock, J.G. 1982. Disease-suppressive soils and root-colonizing bacteria. Science 216:1376-1381.

Shimotsuma, M., Kuc, J., and Jones, C.M. 1972. The effects of prior inoculations with non-pathogenic fungi on Fusarium wilt of watermelon. HortScience 7:72-73.

Shipton, P.J. 1975. Take-all decline during cereal monoculture. In: Biology and Control of Soil-borne Plant Pathogens. Bruehl, G.W., ed., pp. 137-144. American Phytopathological Society, St.Paul, MN. 216 pp.

Shipton, P.J. 1977. Monoculture and soilborne plant pathogens. Ann. Rev. Phytopathol. 15:387-407.

Simon, A., and Sivasithamparam, K. 1988. Microbiological differences between soils suppressive and conducive of the saprophytic growth of Gaumannomyces gramini var. tritici. Can. J. Microbiol. 34:860-864.

Simon, A., and Sivasithamparam, K. 1989. Pathogen-suppression: A case study in biological suppression of Gaumannomyces graminin var. tritici in soil. Soil Biol. Biochem. 21:331-337.

Sleeth, B. 1934. Fusarium niveum, the cause of watermelon wilt. W.Va. Agric. Exp. Stn. Bull. 257:3-23.

Smith, S.N. 1977. Comparison of germination of pathogenic Fusarium oxysporum chlamydospores in host rhizospheres conducive and suppressive to wilts. Phytopathology 67:502-510.

Smith, S.N., and Snyder, W.C. 1972. Germination of Fusarium oxysporum chlamydospores in soils favorable and unfavorable to wilt establishment. Phytopathology 62:273-277.

Sneh, B. 1981. Use of chitinolytic bacteria for biological control of Fusarium oxysporum f. sp. dianthi in carnation. Phytopath. Z. 100:251-256.

Sneh, B., Agami, O., and Baker, R. 1985. Biological control of Fusarium-wilt in carnation with Serratia liquefaciens and Hafnia alvei isolated from rhizosphere of carnation. Phytopath. Z. 113:271-276.

Sneh, B., Dupler, M., Elad, Y., and Baker, R. 1984. Chlamydospore germination of Fusarium oxysporum f. sp. cucumerinum as affected by fluorescent and lytic bacteria from a Fusarium-suppressive soil. Phytopathology 74:1115-1125.

Sneh, B., Pozniak, D., and Salomon, D. 1987. Soil suppressiveness to Fusarium wilt of melon induced by repeated croppings of resistant varieties of melons. J. Phytopathology 120:347-354.

Subba-Rao, N.S., and Bailey, D.L. 1961. Rhizosphere studies in relation to varietal resistance or susceptibility of tomato to Verticillium wilt. Can. J. Bot. 39:1747-1758.

Tamietti, G., and Alabouvette, C. 1986. Résistance des sols aux maladies: XIII. Rôle des Fusarium oxysporum non pathogènes dans les mécanismes de résistance d'un sol de Noirmoutier aux fusarioses vasculaires. Agronomie 6:541-548.

Tamietti, G., and Pramotton, R. 1990. La réceptivité des sols aux fusarioses vasculaires: Rapports entre résistance et microflore autochtone avec référénce particulière aux Fusarium nonpathogènes. Agronomie 10:69-76.

Tennant, D. 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63:995-1001.

Thomashow, L.S., Pierson, L.S., Weller, D.M., and Bonsall, R.F. 1988. Detection of phenazine antibiotics produced by Pseudomonas spp. in soil. (Abstr.) Phytopathology 78:1522.

Thomashow, L.S., and Weller, D.M. 1988. Role of a phenazine antibiotic from Pseudomonas fluorescens in biological control of Gaumannomyces graminis var. tritici. Journal of Bact. 170:3499-3508.

Toussoun, T.A. 1975. Fusarium-suppressive soils. In: Biology and Control of Soil-Borne Plant Pathogens. Bruehl, G.W., ed., pp. 145-150. American Phytopathological Society, St.Paul, MN. 216 pp.

Toussoun, T.A., and Nelson, P.A. 1975. Variation and speciation in the Fusaria. Annu. Rev. Phytopathol. 13:71-82.

Vanderplank, J.E. 1975. Principles of Plant Infection. Academic Press, NY. 216 pp.

Vanderplank, J.E. 1978. Genetic and Molecular Basis of Plant Pathogenesis. Springer-Verlag, NY. 167 pp.

Weller, D.M., and Cook, R.J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.

Weller, D.M., Howie, W.J., and Cook, R.J. 1988. Relationship between in vitro inhibition of Gaumannomyces gramini var. tritici and suppression of take-all of wheat by fluorescent pseudomonads. Phytopathology 78:1094-1100.

Wensley, R.N., and McKeen, C.D. 1963. Populations of Fusarium oxysporum f. sp. melonis and their relation to the wilt potential of two soils. Can. J. Micro. 9:237-249.

Wymore, L.A., and Baker, R. 1982. Factors affecting cross-protection in control of Fusarium wilt of tomato. Plant Dis. 66:908-910.

Yuen, G.Y., and Schroth, M.N. 1986. Inhibition of Fusarium oxysporum f. sp. dianthi by iron competition with an Alcaligenes sp. Phytopathology 76:171-176.

Yuen, G.Y., Schroth, M.N., and McCain, A.H. 1985. Reduction of Fusarium wilt of carnation with suppressive soils and antagonistic bacteria. Plant Disease 69:1071-1075.

BIOGRAPHICAL SKETCH

Robert Philip Larkin was born in Elmhurst, Illinois, on September 10, 1956, to Ronald and Sally Larkin. He attended Bradley University in Peoria, Illinois, and received a Bachelor of Science degree in environmental science-biology in May, 1978. After working for a year as a plant nursery inspector for the Florida Department of Agriculture, he began a graduate program in ecology at the University of Tennessee, Knoxville. He received a Master of Science degree in December, 1981. While working as a biologist at the Central Florida Research and Education Center in Leesburg, Florida, the opportunity arose to enter the doctorate program in plant pathology at the University of Florida, which he began in 1986. Upon completion of the Doctor of Philosophy degree, he will begin post-doctorate work in plant pathology at the University of Missouri. In addition to his professional endeavors, Robert maintains avid interests in music and film.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald L. Hopkins, Chair
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frank N. Martin, Cochair
Assistant Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David J. Mitchell
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H. Corby Kistler
Assistant Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David M. Sylvia
Associate Professor of Soil Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1990

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School